

# Chapter 12



## Biosynthesis of selenium nanomaterials by anaerobic bacteria for environmental technologies

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### 12.1 INTRODUCTION

#### 12.1.1 Nanotechnology for sustainable development

The world is facing formidable challenges in meeting the rising demands for fuels, potable water and consumer products. The health and welfare of people are closely connected to the availability of adequate, safe and affordable energy, water and food supplies (Lens, 2021). Also cost-effective recovery methods of chemicals and resources from waste and wastewater play an increasing role in sustaining human civilization on Earth (Helland & Kastenholz, 2008).

Nanotechnology, comprising technologies working at the scale of typically 100 nm and below, is an enabling technology for a wide variety of traditional scientific disciplines (Fleischer & Grunwald, 2008). This has led to high expectations that nanotechnology will help improve peoples' standard of living, in the short-term by significantly improving existing processes and products, and in the long-term by providing revolutionary and life-changing advances, from cancer treatment and lightweight materials to renewable energy production and (waste)water purification (Lens *et al.*, 2012; Uskokovic, 2007). However, the novel properties that make nanotechnology so interesting have also raised many unanswered questions and concerns related to the impacts, negative and positive, nanotechnology may have on society and the environment (Helland *et al.*, 2006).

### 12.1.2 Nanotechnology and water treatment

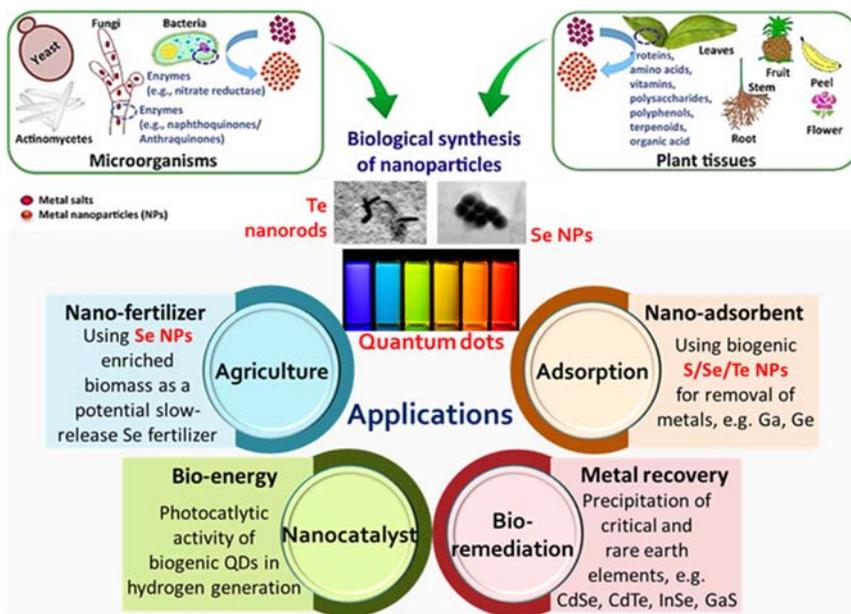
Nanotechnology encompasses the creation and utilization of materials, devices and systems at the level of atoms and molecules, cutting across disciplines such as chemistry, physics, biology, engineering and material science (Masciangioli & Zhang, 2003). Nanomaterials often exhibit novel and significantly changed physical, chemical and biological properties. These are the result of their structure, larger surface area per unit volume and quantum effects that occur at the nanoscale. For environmental technology, the potential impact areas comprise three categories: treatment and remediation, sensing and detection, and prevention (Theron *et al.*, 2008).

Within the category of treatment and remediation, nanotechnology has the potential to contribute to long-term availability and viability of water resources of high quality (Bottero *et al.*, 2006), through the use of advanced filtration materials (nanostructured and nanoreactive membranes), bioactive nanoparticles for water disinfection (silver or titanium dioxide (TiO<sub>2</sub>) nanoparticles) and engineered nanoparticles and nanomaterials that remove pollutants (e.g., carbon nanotubes sorbing heavy metals or organics; nanoparticles with enhanced catalytic properties that degrade organic matter, including nanoscale semiconductor photocatalysts, single-enzyme and zero-valent iron nanoparticles) (Zhang, 2003). Figure 12.1 illustrates different application areas of selenium (and other chalcogen) based nanomaterials for environmental technology and resource recovery.

### 12.1.3 Nanobiomanufacturing of nanomaterials

As the future of materials science is closely linked to nanotechnology, there is a need to improve the manufacturing of nanomaterials in terms of its environmental and economic impact. Nanoproducts can be prepared by different methods, such as colloidal aqueous and micellar solution synthesis methods, using ultrasonic waves, microwaves or gamma-irradiation (Yong *et al.*, 2003). In most cases, particles prepared by these methods have some problems including poor reproducibility, control of particle size, distribution and shape (Mandal *et al.*, 2005). Some reactions require high temperature, and/or high pressure for initiating the reaction, and/or inert atmosphere protection, and/or using toxic matters such as H<sub>2</sub>S, toxic templates and stabilizers, and metallic precursors (Gericke & Pinches, 2006; Yong *et al.*, 2003). In addition, nanomaterial production has to be competitive, and the high costs associated with many existing processes limit the development and transfer of new nanotechnologies into the marketplace.

Thus, there is an increasing interest in the development of environmentally clean synthetic procedures for the formation of nanoproducts. An environmentally acceptable solvent system as well as eco-friendly reducing and capping agents



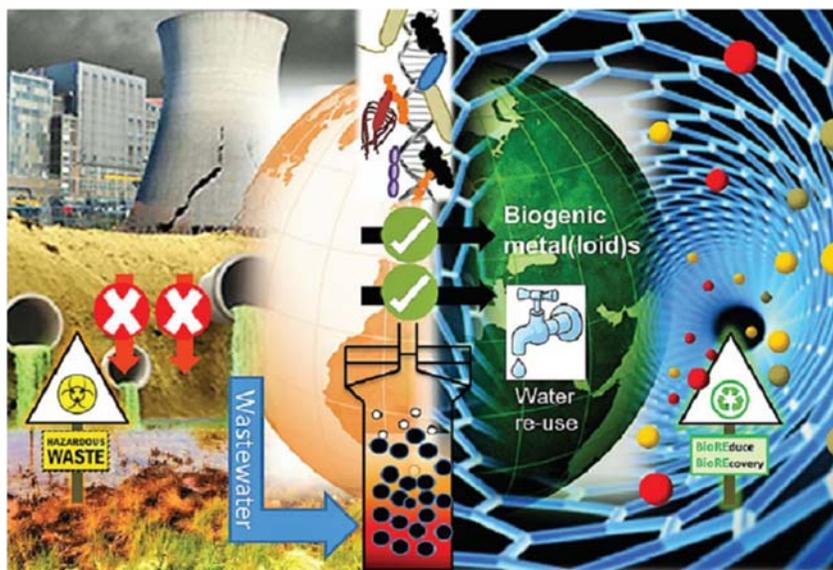
**Figure 12.1** Biosynthesis and applications of chalcogen nanoparticles. Agricultural aspects are further detailed in Chapters 8 and 9, some nanocatalytic properties of selenium nanoparticles are detailed in Chapter 11.

are essential elements for a green nanoparticle synthesis. A biological approach to materials synthesis satisfies these criteria and is of benefit to the materials manufacturing industry. Using the capability of bacterial cells to manufacture useful bioinorganic offers a revolutionary method of materials synthesis that eliminates toxic organic solvents, minimizes expensive high-temperature processing and can involve the use of industrial waste as the starting material. Figure 12.2 illustrates the concept of the biomanufacturing of inorganic nanoparticles from metal and metalloid contaminated wastewaters using the anaerobic bacteria present in the granular sludge of an upflow anaerobic sludge bed (UASB) reactor, a reactor type commonly used for wastewater treatment (Jacob *et al.*, 2016; Mal *et al.*, 2017a).

## 12.2 THE BIOGEOCHEMICAL SELENIUM CYCLE

### 12.2.1 The element selenium and environmental pollution by selenium

Selenium (Se) belongs to the periodic table group 16 (together with oxygen (O), sulfur (S), and tellurium (Te)), which are called chalcogens. Se is called



**Figure 12.2** Illustration of the production of functional nanoparticles from selenium and/or metal contaminated wastewater using anaerobic bacteria present in the commonly used upflow anaerobic sludge bed (UASB) wastewater treatment reactors.

an ‘essential toxin’, as it is required for certain cell processes and enzymes, but becomes toxic at greater doses (Stolz *et al.*, 2006). Se is a key trace element found in representative species from all three domains of life: *Bacteria*, *Archaea* and *Eukaryota* (Oremland *et al.*, 2004; Sharma & Singh, 1984).

Generally, selenium occurs at trace levels in the environment. However, in a few areas, selenate ( $\text{SeO}_4^{2-}$ ) or selenite ( $\text{SeO}_3^{2-}$ ) concentrations are appreciable and selenium becomes toxic to bacteria or the animals drinking these waters (Ohlendorf, 2002; van Fleet-Stalder *et al.*, 2000). Anthropogenic activities interfere with the global selenium cycle influencing it crucially. It has been estimated that 35–40% of the total selenium emissions to the atmosphere are due to anthropogenic activities (Wen & Carignan, 2007), including foremost the combustion of coal and oil, non-ferrous metal melting and utilization of agricultural products (Lenz & Lens, 2009). Agricultural drainage waters, oil refining wastewaters and coal combustion residues contaminate the lotic, lentic and marine environment. Commonly, selenium oxyanion contamination occurs concomitantly with sulfate in different waste streams (Table 12.1). Selenium is introduced in the terrestrial compartment mainly as fertilizer or by mining activities.

**Table 12.1** overview of sulfate and selenium concentrations in selenium contaminated waste streams.

Waste stream	SeO <sub>4</sub> <sup>2-</sup> [mg L <sup>-1</sup> ]	Se [μg L <sup>-1</sup> ]	Reference
Acid mine drainage	1880	16	<a href="#">España <i>et al.</i> (2006)</a>
Acid mine drainage	1146	492	<a href="#">Simmons <i>et al.</i> (2002)</a>
Acid seeps (Moreno shale, USA)	12,500	420	<a href="#">Presser (1994)</a>
Flue gas desulfurization purge waters	815	536	<a href="#">Cantafio <i>et al.</i> (2001)</a>

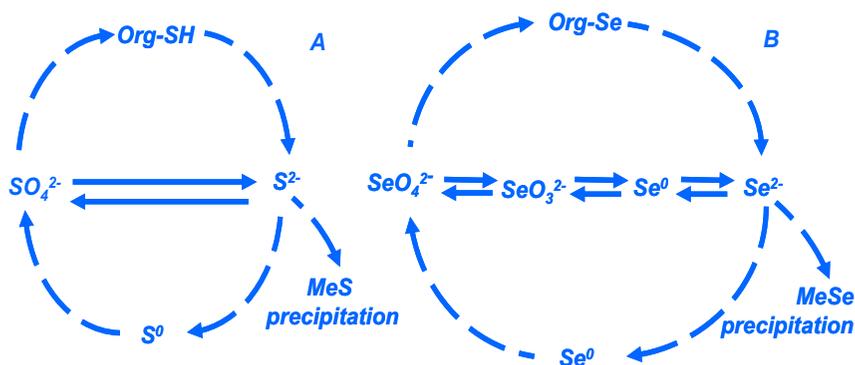
### 12.2.2 Microbial Se metabolism

Although bioreduction of selenium oxyanions (SeO<sub>4</sub><sup>2-</sup> and SeO<sub>3</sub><sup>2-</sup>) to Se<sup>0</sup> or selenide (Se<sup>2-</sup>) by anaerobic microorganisms is an important part of the biogeochemical selenium cycle, it has thus far been rather poorly studied compared to the carbon, nitrogen, phosphorus and sulfur cycles. The selenium cycle is driven by prokaryotes ([Chasteen & Bentley, 2003](#); [Dungan & Frankenberger, 1998](#)), which readily metabolize selenium for a range of metabolic functions including assimilation, methylation, detoxification and anaerobic respiration ([Oremland \*et al.\*, 2004](#)).

Microbes reduce Se(IV) or Se(VI) for a number of different reasons, including detoxification and energy conservation (see Chapter 3). For example, in *Bacillus selenitireducens*, the Se(IV) (e.g., SeO<sub>3</sub><sup>2-</sup>) reduction mechanism involves energy conservation by oxidation of lactate coupled to growth via respiratory reduction of Se(IV) using Se-specific dissimilatory enzymes ([Blum \*et al.\*, 1998](#)). Fe(III) reducers, for example, *Shewanella oneidensis* ([Klonowska \*et al.\*, 2005](#)) and *Geobacter sulfurreducens* ([Pearce \*et al.\*, 2008](#)), can also reduce Se(IV) with c-type cytochromes implicated in electron transfer to the metalloid. Unlike *Shewanella* and *Geobacter* species, *Veillonella atypica* does not possess high concentrations of cytochromes and produces Se<sup>0</sup> nanospheres from Se(IV) via a hydrogenase-coupled reduction, mediated by ferredoxin.

### 12.2.3 Microbial synthesis of Se nanoparticles

Over the past three decades, the finer aspects of the selenium biogeochemical cycle have begun to emerge. The dissimilatory reduction of soluble SeO<sub>4</sub><sup>2-</sup> via SeO<sub>3</sub><sup>2-</sup> to insoluble elemental selenium (Se<sup>0</sup>) has been shown to be a significant and rapid environmental process ([Lloyd & Lovley, 2001](#)). A particular feature of selenium is that Se<sup>0</sup> can be formed in a single reduction step ([Figure 12.3](#)), in contrast to elemental sulfur (S<sup>0</sup>) which requires a two-step process (complete reduction to sulfide followed by partial oxidation to S<sup>0</sup>). Environmental conditions (e.g., pH,



**Figure 12.3** Comparison of the biological sulfur (a) and selenium (b) cycles.

temperature and salinity) and (waste)water geochemistry (electron donors and acceptors) influence the biochemical production and excretion of  $\text{Se}^0$ , as well as the physical and chemical properties of the  $\text{Se}^0$  nanospheres. There is still much unknown regarding the factors that determine the bioreduction end product:  $\text{Se}^0$ , selenide or a mixture of oxidation states, and thus the possibility for metal selenide precipitation.

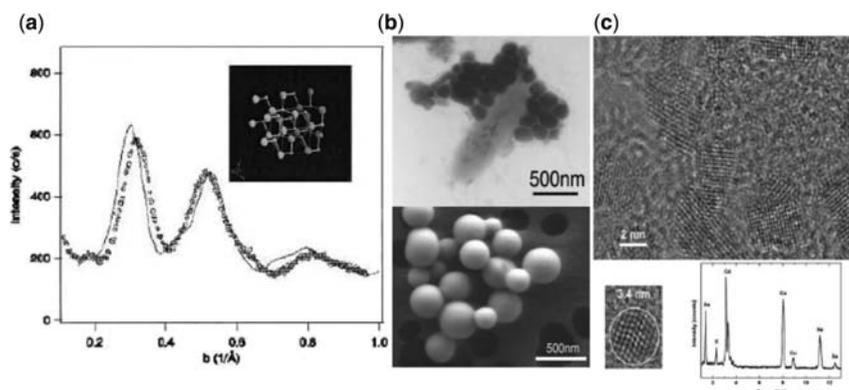
## 12.3 CHARACTERISTICS OF SELENIUM NANOPARTICLES

The discovery that selenium-respiring bacteria form  $\text{Se}^0$  (Oremland *et al.*, 2004) and metal selenide (Pearce *et al.*, 2008) nanospheres offers an appealing technological potential. Indeed, these nanoparticles possess optical and semiconducting properties that are highly desired for nanotechnological applications (Figure 12.4).

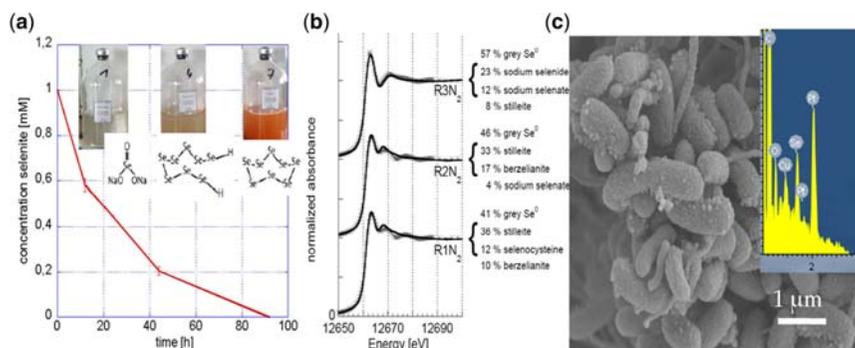
### 12.3.1 Elemental ( $\text{Se}^0$ ) nanoparticles

$\text{Se}^0$  particles can be formed intracellularly, extracellularly or on the cell surface. The  $\text{Se}^0$  exospheres have a uniform diameter (0.2–0.3  $\mu\text{m}$ ). They eventually slough off the cell surface into the medium (Gonzalez-Gil *et al.*, 2016), from which they can be harvested and further processed. Lenz *et al.* (2008) showed that the  $\text{Se}^0$  nanoparticle diameter and zeta potential is electron donor (acetate or hydrogen) dependent. Moreover, X-ray absorption near edge structure spectroscopy (XANES) showed that the nanospheres not only contain native  $\text{Se}^0$ , but also various selenide compounds (Figure 12.5).

Biogenic  $\text{Se}^0$  nanospheres formed by *S. barnesii*, *B. selenitireducens* and *S. shriftii*, when grown with selenium oxyanions, were found to have unusual photo-optical and semiconducting physical properties with potential industrial



**Figure 12.4** Examples of biosynthetic chalcogen nanomaterials with technological potential. (a) wide-angle X-ray scattering (WAXS) of *Schizosaccharomyces pombe* CdS nanoparticles, proposed to be used in the fabrication of diodes (Kowshik *et al.*, 2002a, 2002b, 2003), (b) Transmission electron microscopy (TEM, top) and scanning electron microscopy (SEM, bottom) of  $\text{Se}^0$  produced by *S. barnesii*, with potential applications in photocopiers and microelectronic circuits (van Fleet-Stalder *et al.*, 2000) and (c) TEM micrographs and energy-dispersive X-ray (EDX) spectra of size-fractionated 2-mercaptoethanol-stabilized CdSe quantum-dots, produced using biologically produced  $\text{Se}^{2-}$  by  $\text{SeO}_3^{2-}$  fed *V. atypica* (Pearce *et al.*, 2008). *V. atypica* also excreted ZnSe and CdSe nanoparticles, but these were too large (30 nm) for quantum-dot type applications.



**Figure 12.5** Microbiologically formed  $\text{Se}^0$  exospheres obtained by the reduction of sodium selenite (1 mM) by a mixed culture of anaerobic microorganisms growing in a wastewater treatment bioreactor: color changes in the medium coupled to structural changes of the  $\text{Se}^0$  (a), K-edge Fourier transforms of  $\text{Se}^0$  precipitates (b), and scanning electron micrograph with energy dispersive X-ray analysis of Se exospheres (c) (from Lenz *et al.*, 2008).

applications in devices such as photocopiers and microelectronic circuits (Oremland *et al.*, 2004). Moreover, Dixit *et al.* (2021) described the magnetic properties of biogenic selenium nanomaterials produced by anaerobic granular sludge and elaborated on their potential application in magnetic refrigeration. Jain *et al.* (2015a, 2017) further showed biogenic Se<sup>0</sup> nanoparticles have a high sorption capacity for divalent cations, for example, Zn and Cu, and can thus be used as nanosorbents. Biogenic Se<sup>0</sup> can also be used as biofertilizer, either as soil amendment or as foliar application (Li *et al.*, 2021; see also chapter 9).

### 12.3.2 Metal selenide nanoparticles

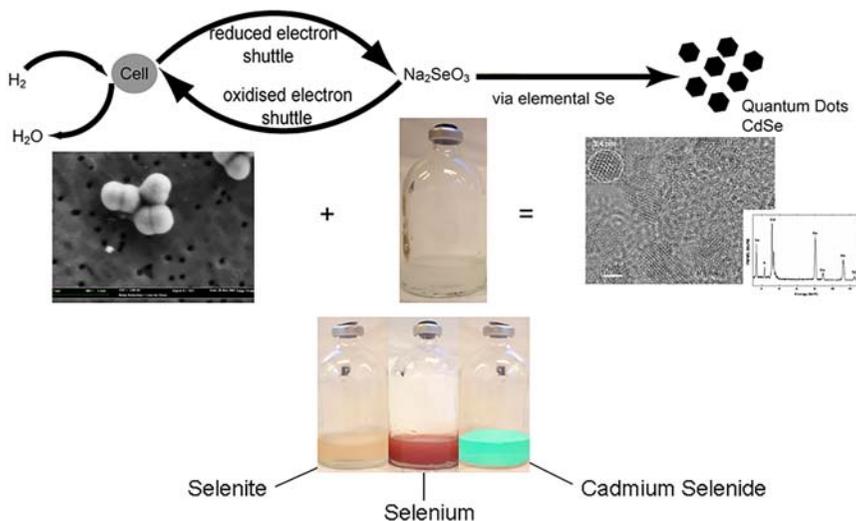
A variety of regular spherical and non-spherical nanocolloids (including wires, tubes, disks, and more exotic structures like prisms and branched structures) of metal chalcogenides have been synthesized (Shanbhag *et al.*, 2007). These nanocolloids have been prepared from materials such as CdS, CdSe, CdTe, PbS, PbSe and ZnSe through a number of synthetic and biological routes (Cho *et al.*, 2005; Cozzoli *et al.*, 2005).

Chalcogen quantum-dots (QDs) are semiconducting crystals comprised of sulfur (S), selenium (Se) or tellurium (Te) in combination with transition metals, to form compounds such as zinc sulfide (ZnS), zinc selenide (ZnSe), cadmium selenide (CdSe), lead selenide (PbSe) and cadmium telluride (CdTe). QDs are prepared from a range of organic, inorganic and organometallic materials via a number of synthetic routes. As a result of the production process, QDs are coated with an organic capping agent. However, inorganic passivating agents can also be used to produce core/shell-type structures with shells formed of chalcogen compounds, for example, CdSe/CdS, CdSe/ZnS, ZnSe/ZnS, and a range of other materials, for example, composite spheres of silica and CdSe nanocrystals. As with the regular spherical QDs, the morphology of these nanocrystals can be varied, with non-spherical structures including wires, tubes, disks, and more exotic structures like prisms and branched structures.

There is a lot of excitement in the scientific community about the chemical, optical and electronic properties of such ‘quantum-confined’ structures, governed by their chemical composition, size and shape. Despite this excitement, there remain many unanswered questions about the mechanisms of formation of such complex nanoscale systems and the possibility of achieving these through advancements in bio-engineering to develop an industrial biotechnological process. However, initial results have highlighted the potential for using a novel biological approach in an environmentally friendly, aqueous-based synthesis to precipitate nanoscale, luminescent, semiconducting CdSe and ZnSe QD (Figure 12.6).

### 12.3.3 Toxicity of selenium nanoparticles

The implementation of selenium based nanoparticles (e.g., CdSe, CdTe, and PbSe) is, however, hindered by the presence of toxic elements, like Cd or Pb.



**Figure 12.6** Microbial synthesis of chalcogenide-based nanoparticles via the reduction of sodium selenite using the metal-reducing bacterium *Veillonella atypica* (from Pearce *et al.*, 2008).

Technological applications thus also need to address the potential toxicity of this type of quantum-dots. This is of concern primarily because many quantum-dot core metals, such as zinc, cadmium, lead and selenium are toxic at relatively low concentrations (parts per million) and thus of considerable human health and environmental concern (Hardman, 2006; Chapter 1). Little information is available on routes of exposure to quantum-dots, their stability, aerosolization, and how they partition into different environmental compartments (e.g., soil, sediment, water, air or biota). Environmental exposure could result from, for example, leakages and spillage during manufacturing and transport (Zhang *et al.*, 2019). The toxicological properties of selenium as well as these metals in the form of nanoparticles may differ substantially from the bulk materials, but are nonetheless clearly of concern.

Hardman (2006) reviewed studies on the toxicity of quantum-dots. While some studies in cell culture systems (*in vitro*) have shown that quantum-dots are not toxic to cells, others have shown they can have toxic effects. Overall, these studies showed that toxicity depends on many factors, including the physicochemical properties (size, electrical charge, concentration, outer coating bioactivity and stability) of the quantum-dots and environmental conditions (Hardman, 2006). The main reasons for the cytotoxicity of CdSe/CdTe quantum-dots are desorption of Cd (i.e., QD core degradation), free radical formation, and interaction with intracellular components or bioavailability (uptake) of QDs. Biogenic nano- $Se^0$  synthesized by anaerobic granular sludge was 10-fold less

toxic than chemically synthesized nano-Se<sup>0</sup> (Mal *et al.*, 2017c). This indicates that the presence of extracellular polymeric substances (EPS) increases the physiochemical stability of biogenic nano-Se<sup>0</sup> and prevents their dissolution. The presence of EPS on the surface of biogenic nano-Se<sup>0</sup> plays a major role in lowering the bioavailability (uptake) and toxicity of Se<sup>0</sup> nanoparticles. Still, detailed studies are required on the toxicity of biogenic CdSe/CdTe nanoparticles and the role EPS plays in it. It is also important to focus on biological synthesis of 'Cd-free' QDs (e.g., CuSe/CuTe) in the near future (Xu *et al.*, 2016; Supreet & Singh, 2020).

## 12.4 NANOSORBENTS

Biogenic Se<sup>0</sup> nanospheres have adsorptive properties with considerable technological potential. For instance, Johnson *et al.* (2008) reported that unstabilized nano-selenium in dry powder as well as in impregnated cloth was successful for the *in situ*, real time suppression of mercury (Hg) vapor escape following the fracture of compact fluorescent lamps. Nano-selenium was surprisingly much more effective in capturing Hg than sulfur (micro-sulfur and sulfur nanotubes), metals (micro- and nano-particles of zinc, nickel, copper and silver) and carbon (black carbon and activated carbon) nanoparticles. This unknown feature of Se<sup>0</sup> was attributed to the extremely high affinity of Se for mercury and the strong Hg/Se binding (Ralston *et al.*, 2007). Again, the efficiency of biogenic Se<sup>0</sup> needs to be demonstrated, but it is highly likely that similar Hg removal efficiencies can be obtained.

### 12.4.1 Metal adsorption onto biogenic nano-Se<sup>0</sup>

The adsorption of Zn, as a model divalent heavy metal, onto biogenic nano-Se<sup>0</sup> produced by anaerobic granular sludge (Jain *et al.*, 2015a) and fungal *Phanerochaete chrysosporium* pellets (Espinosa-Ortiz *et al.*, 2016) has been investigated. The adsorption of Zn onto biogenic nano-Se<sup>0</sup> at acidic pH values follow a ligand-like (type II) adsorption mechanism (Jain *et al.*, 2015a). The adsorption of Zn onto biogenic nano-Se<sup>0</sup> followed a two-step process at near-neutral pH. X-ray photoelectron spectroscopy (XPS) suggested the precipitation of one of the Zn species which could have been ZnO, ZnSe, Zn(OH)<sub>2</sub>, or ZnCO<sub>3</sub>. Preliminary extended X-ray absorption of fine structure (EXAFS) data analysis suggests the absence of ZnSe, thus discarding the occurrence of disproportionation of elemental selenium. The ζ-potential of biogenic nano-Se<sup>0</sup> became less negative at higher loading of Zn, resulting in lower colloidal stability, thus leading to higher retention of biogenic nano-Se<sup>0</sup> on the filter when compared to biogenic nano-Se<sup>0</sup> after filtration but without adsorption (Jain *et al.*, 2015a).

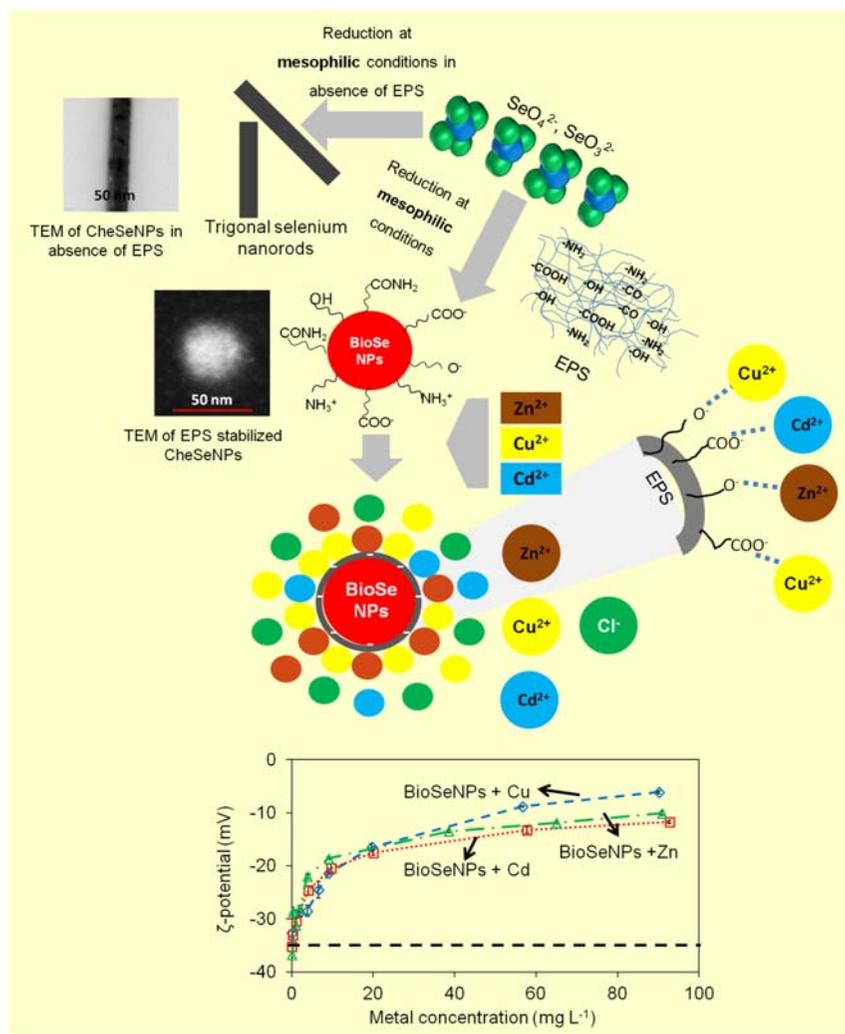
The selective adsorption of heavy metals onto biogenic nano-Se<sup>0</sup> was explored by Jain *et al.* (2016a, b). It was found that the metals to biogenic nano-Se<sup>0</sup> ratio

(v:v) and pH can be manipulated to optimize the selective adsorption of Cu. At the metal to biogenic nano-Se<sup>0</sup> ratio of 1:1 (v:v) and theoretical pH of 4.3, Cu was found to adsorb 4.7 times more onto biogenic nano-Se<sup>0</sup> than the total sum of Cd and Zn adsorbed, when an equimolar mixture of Cu, Cd and Zn was used in the adsorption experiments. The selective preference of Cu onto biogenic nano-Se<sup>0</sup> depends on the intrinsic properties of Cu: smaller ionic radius, higher electronegativity, higher ratio of ionization potential to ionic radius and higher first stability constant of metal hydroxo species and acetate complexes (Jain *et al.*, 2016b). The selective preference of Cu also depends on the presence of functional groups such as hydroxyl and carboxyl on the surface of biogenic nano-Se<sup>0</sup>. Indeed, Fourier-transform infrared spectroscopy (FT-IR) analysis of biogenic nano-Se<sup>0</sup> loaded with heavy metals confirmed the interaction of hydroxyl and carboxyl groups on the surface of biogenic nano-Se<sup>0</sup> with heavy metals (Jain *et al.*, 2016b).

#### 12.4.2 Role of EPS in the fate of selenium in the environment and bioreactors

Figure 12.7 demonstrates the role of the extracellular polymeric substances (EPS) in the formation and characteristics of biogenic nano-Se<sup>0</sup>. EPS is mainly composed of polysaccharides, proteins, humic substances, lipids and nucleic acids (D'Abzac *et al.*, 2010; Sheng *et al.*, 2010). Proteins and polysaccharides are the major components of EPS. Generally, EPS is known to retard or prevent the dispersion of nanomaterials such as silver nanoparticles (Kang *et al.*, 2014; Tourney & Ngwenya, 2014). In contrast, Jain *et al.* (2015b) showed that the EPS provides colloidal stability to the biogenic nano-Se<sup>0</sup> due to less negative  $\zeta$ -potential values (Buchs *et al.*, 2013; Dhanjal & Cameotra, 2010; Jain *et al.*, 2015a). Bare elemental selenium has been reported to have a  $\zeta$ -potential of  $-10$  mV as compared to  $-30$  mV observed for biogenic nano-Se<sup>0</sup> (Dhanjal & Cameotra, 2010; Jain *et al.*, 2015a). This colloidal stability of the biogenic nano-Se<sup>0</sup> is the reason for their presence in bioreactor effluents (Lenz *et al.*, 2008) as well as the high mobility of biogenic nano-Se<sup>0</sup> in the environment (Buchs *et al.*, 2013).

The presence of EPS on the surface of biogenic nano-Se<sup>0</sup> determines the mechanism of interaction of the biogenic nano-Se<sup>0</sup> with heavy metals. The capture of mercury from the vapor phase by elemental selenium is due to the precipitation of mercury selenide on the surface of the elemental selenium (Fellowes *et al.*, 2011; Johnson *et al.*, 2008). However, the interaction of Zn with biogenic nano-Se<sup>0</sup> does not lead to formation of ZnSe as observed by Jain *et al.* (2015a). The interaction of heavy metals with biogenic nano-Se<sup>0</sup> is essentially an interaction of heavy metals with the EPS layer present on the surface of the biogenic nano-Se<sup>0</sup>. Indeed, EPS is known to interact with heavy metals (D'Abzac *et al.*, 2010). The presence of amine and carboxylate groups on the surface can lead to the adsorption of heavy metals by ligand-like (Type II) adsorption, as



**Figure 12.7** Summary of the role of EPS in determining the properties of the biogenic nano-Se<sup>0</sup> (Jain, 2014).

observed during the adsorption of Cu on cellulose modified with poly(glycidyl methacrylate) and polyethyleneimine (Navarro *et al.*, 2001). The presence of these surface groups on biogenic nano-Se<sup>0</sup> was confirmed by XPS and FT-IR analyses (Jain *et al.*, 2015a, b). Thus, the presence of such a layer of EPS on biogenic nano-Se<sup>0</sup> is responsible for ligand-like (Type II) adsorption of Zn onto biogenic nano-Se<sup>0</sup> at acidic pH (Jain *et al.*, 2015a). The presence of hydroxyl and carboxyl groups on the surface of biogenic nano-Se<sup>0</sup> can be attributed to the

presence of EPS. The FT-IR data confirm that these groups interact with the heavy metals. The presence of these groups and the higher first stability constant of metal hydroxo and metal acetate complexes for Cu lead to a higher preference of biogenic nano-Se<sup>0</sup> towards Cu (Jain *et al.*, 2015a; Sitko *et al.*, 2013). Thus, the presence of EPS on the surface of biogenic nano-Se<sup>0</sup> is further affecting the affinity of the biogenic nano-Se<sup>0</sup> towards different heavy metals.

## 12.5 PHOTONIC NANOCRYSTALS AND PHOTOCATALYSTS

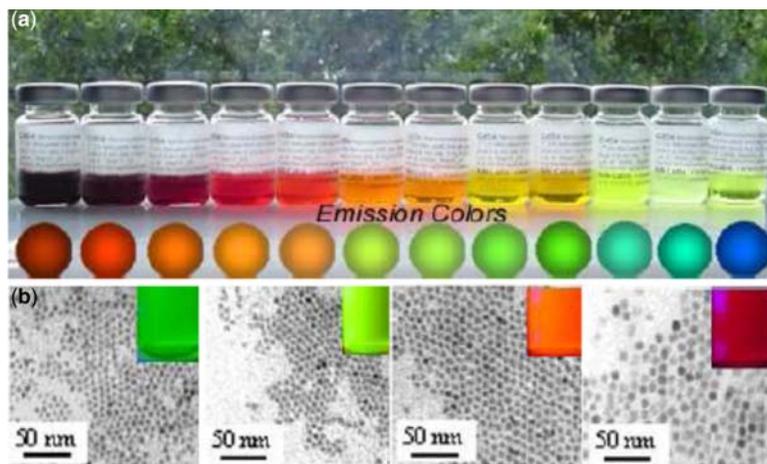
### 12.5.1 Photonic colloidal nanocrystals – quantum-dots

In recent years, nanoscience and nanotechnology have brought a great revolution in different areas (Grim *et al.*, 2015). In particular, the synthesis of transition metal nanoparticles has been of great relevance for their use in areas such as biomedicine (see Chapter 10), antimicrobial properties or catalytic applications for chemical synthesis (see Chapter 11). A particular class of these materials are colloidal quantum-dots (QDs), solution-processed semiconductor nanocrystals of 2–10 nanometers (10–50 atoms) in size. QDs have unique properties because, at this size, they behave differently to their bulk equivalents and exhibit unprecedented tunability, enabling completely new applications in science and technology (see Chapter 11). They are particularly suited to initiate a future generation of photonic devices (Figure 12.4).

Most of the research interest regarding semiconducting QDs has been focused on their electrical conductivity and unique optical (Figure 12.8) properties, which can be greatly altered and controlled by an external stimulus (e.g., voltage or photon flux), making them critical components of many different kinds of electrical circuits and optical applications. The properties of quantum structures are strongly dependent on their size and shape, and the quality of their internal crystal structure (e.g., structural defects) can serve as trapping sites for electrons or holes.

### 12.5.2 Biomanufacturing of quantum-dots using bacteria during wastewater treatment

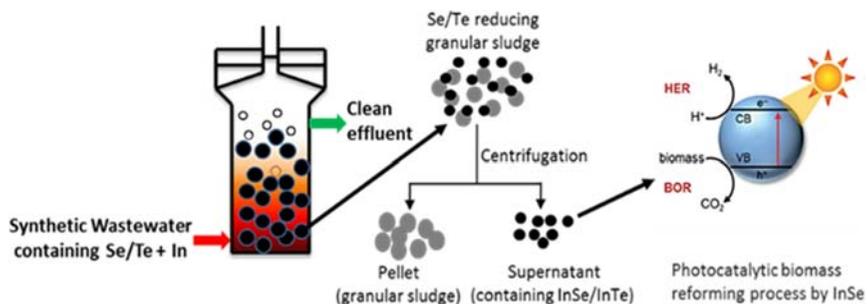
Strategies in QD synthesis focus on the development of methodologies that allow the creation of these nanomaterials in a simple, efficient and sustainable way (Sakimoto *et al.*, 2017). For this reason, researchers have started to apply biological entities as an elegant approach for the direct synthesis of metal chalcogen nanoparticles. Microorganisms have the ability to induce the formation of QDs, as outlined in Section 12.3.2, sometimes even controlling the size and structural shape, and avoiding aggregation problems (Xu *et al.*, 2016). Figure 12.6 illustrated this microbial synthesis route, based on the precipitation of the divalent metal and the microbially formed selenide (Figure 12.3(b)).



**Figure 12.8** Commercially available quantum-dots, prepared via chemical synthesis. (a) 12 Wavelength CdSe Kit of quantum-dots with different emission wavelengths between 480 and 640 nm and (b) TEM micrographs with corresponding emission of quantum-dot aliquots, marketed by Nanomaterials & Nanofabrication Laboratories (Image from [www.nn-labs.com](http://www.nn-labs.com)).

Metal ions such as cadmium or zinc present in the selenium-rich wastewaters influence the microbial reduction and the fate of biogenic Se(0) nanoparticles (Mal *et al.*, 2016a). When metal ions are available, they can either form metal-selenide nanoparticles after reacting with selenide ( $\text{HSe}^-$ ) generated during the microbial reduction of Se-oxyanions or they form metal-Se(0) complexes due to adsorption of metals onto Se(0) nanoparticles (Jain *et al.*, 2016b; Mal *et al.*, 2016b). Mal *et al.* (2017a) showed that microbial reduction of selenite (Se(IV)) in the presence of Cd(II) by anaerobic granular sludge results in the formation and deposition of cadmium selenide (CdSe) nanoparticles by the reaction of Cd (II) with biogenic  $\text{HSe}^-$ , but also Se(0) nanoparticles and Se(0)-Cd complexes can be formed. In further research, Wadgaonkar *et al.* (2018) showed that mixed microbial cultures present in anaerobic wastewater treatment reactors can also form selenium (CdSe, ZnSe and PbSe) as well as tellurium (CdTe and ZnTe) based nanoparticles, which are capped by a layer of extracellular polymers (Figure 12.9).

Little is known about the mechanism of metal selenide nanoparticle formation and their recovery from a bioreactor. The principles of nucleation, crystal growth and agglomeration also apply to metal selenide formation and some of the processes described for metal sulfide formation can be adopted to metal selenide formation. For the former, the focus is more on the formation of large, well-settling crystals of metal sulfides of base metals (e.g., ZnS, CuS, NiS, CoS, CdS and PbS) for efficient bioseparation (Esposito *et al.*, 2006). Metals are



**Figure 12.9** Schematic representation of microbial synthesis of chalcogenide-based nanoparticles using anaerobic granular sludge in an upflow anaerobic granular sludge bed (UASB) bioreactor. Thus, nanoparticles of ZnS (Jacob *et al.*, 2020), elemental selenium ( $\text{Se}^0$ ; Lenz *et al.*, 2008), CdSe (Mal *et al.*, 2016b), elemental tellurium (Mal *et al.*, 2017b) and CdTe (Wadgaonkar *et al.*, 2018) have been synthesized. Mal *et al.* (2020) showed the nanoparticles are mainly present in the loosely bound fraction of the EPS, and can thus be harvested by centrifugation of the sludge. Most reactors were operated at mesophilic (30°C) temperatures, with a few studies investigating psychrophilic (Zeng *et al.*, 2019) and thermophilic (Dessi *et al.*, 2016) conditions. BOR: biomass oxidation reaction; HER: hydrogen evolution reaction.

typically separated or concentrated as sulfides using  $\text{Na}_2\text{S}$ ,  $\text{NaHS}$  or  $\text{H}_2\text{S}$  (Veeken *et al.*, 2003). Biogenic sulfide produced by sulfate reducing bacteria (SRB) is an interesting alternative sulfide source, especially when besides metals also sulfate is present in the wastewater, for example, wastewaters from metal refineries and acid mine drainage (White & Gadd, 1999). Metal sulfides have very low solubility products, and nucleation thus occurs much faster than crystal growth, so that nanoparticles and fines of many metals, for example, CdS, ZnS and PbS, are also formed during bioprecipitation (Klaus-Joergler *et al.*, 2001). Similarly, Jacob *et al.* (2020) showed ZnS nanoparticles can be formed using sulfate reduction by anaerobic granular sludge. The CdSe nanoparticles are present in the loosely bound fraction of the EPS of anaerobic granules, and can thus be removed from the granular sludge by centrifugation (Mal *et al.*, 2020). In some cases, however, metal sulfide formation can result in a complete encrustation of the sulfate reducing cell (Jiang *et al.*, 2014) and it can be preferred to produce the nanoparticles in a separate reactor, downstream of the sulfide or selenide producing bioreactor. Thus, König *et al.* (2006) developed dynamic modeling and feedback control of the ZnS precipitation process. A dynamic mass balance model for ZnS precipitation was developed and an adequate strategy for controlling the sulfide (using a pS-electrode) and pH-level in a continuous flow stirred tank reactor (CFSTR) was designed in order to create appropriate conditions for precipitation, independent of the conditions in the sulfide producing bioreactor. A similar approach could be developed for the production

of metal selenide nanoparticles, using biologically produced selenide in an upfront bioreactor.

### 12.5.3 Photocatalytic processes using chalcogen nanoparticles

In recent years, metal sulfide and selenide nanoparticles, particularly ZnS and CdS, have been intensively studied as active photocatalysts in virtue of their unique catalytic functions (Qin *et al.*, 2011). ZnS is a promising photocatalyst for degradation of organic pollutants (Jacob *et al.*, 2020), photoreduction of CO<sub>2</sub> (Meng *et al.*, 2017) and H<sub>2</sub> production (Kuehnel & Reisner, 2018) because of the rapid generation of electron-hole pairs by photoexcitation and highly negative reduction potentials of excited electrons (Chen *et al.*, 2013; Qin *et al.*, 2011). However, the bandgap of ZnS is 3.66 eV (Yoneyama, 1997), which is too large for visible light response. Doping with metal ions and combining with various narrow bandgap semiconductors have been applied to make ZnS have visible light activity. Ni, Cu and Pb-doped ZnS showed visible-light photocatalytic activities for H<sub>2</sub> production (Chen *et al.*, 2013).

Although many inorganic semiconductors have been used for photocatalytic CO<sub>2</sub> conversion, the performance of most of these materials is still rather sluggish due to the low conduction band level and/or poor activation ability of CO<sub>2</sub> molecules (Tu *et al.*, 2014). A promising strategy is to couple the inorganic semiconductor with an organic metal complex as a photosensitizer/electrocatalyst to achieve a more efficient reaction rate (Meng *et al.*, 2017; Sekizawa *et al.*, 2013). The drawback of these hybrid photocatalysts is that they generally need to be operated in an organic medium (e.g., acetonitrile or alcohol) in the presence of organic sacrificial agents (e.g., triethanolamine or alcohol). Therefore, a reaction system that can achieve efficient and stable CO<sub>2</sub> conversion is urgently needed. Chalcogen based nanoparticles, in particular metal selenides, are good candidates for this.

### 12.5.4 Nanobiohybrids

#### 12.5.4.1 Microorganism – quantum-dot nanohybrids

Nanobiohybrids are a nanoscale combination of engineered nanomaterials with bioactive substances (Palomo, 2019). Various kinds of components can be utilized in preparation of nanobiohybrids. Among them, naturally obtained materials, for both the nano and bio part, are gaining interest because of their eco-friendliness and bio-compatibility (Chinnaiyan *et al.*, 2019). In order to maximize applicability of nanobiohybrids, it is important to develop various nanomaterials with tailor-made physico-chemical properties as well as to control the interactions between the nanomaterials and bio-component of the hybrids.

An important issue is the selection of the biological entity of the nanohybrid. One of the main strategies described in the literature for this green synthesis approach is

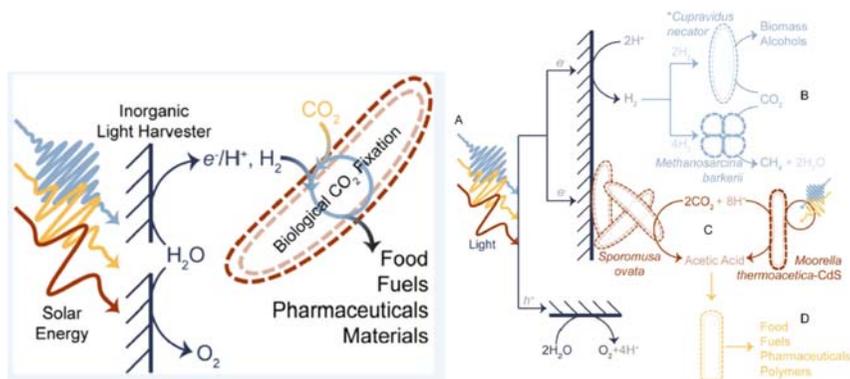
based on the use of microorganisms (Jacob *et al.*, 2016). Prokaryotic or eukaryotic microorganisms have been employed for the preparation of nanoparticles of different metals (Au, Ag, Cd, Pt, Zn and Fe<sub>3</sub>O<sub>4</sub>) under moderate pressures and temperatures. The advantage is that microorganisms secrete large quantities of enzymes, which participate in the enzymatic reduction of metal ions (Nancharaiah & Lens, 2015). The localization and morphology of the nanoparticles depend on the microbial species because of the different enzymes and proteins that can be involved in the biomineralization, generating different sizes of nanoparticles.

One of the candidates for the nano-part of bacterial nanohybrids are quantum-dots (Sakimoto *et al.*, 2017). Quantum-dots are semiconductor nanocrystals with diameters less than 100 nm, and are composed of heavy metals (Cd, Zn, ...) and chalcogens (S, Se or Te) (Xu *et al.*, 2016; see section 12.5.1). Because of their very specific light spectra, they are particularly suited to initiate a future generation of photonic devices. The discovery of the quantum confinement effect, now just over 30 years ago, and the first organic 'hot-injection' quantum-dot synthesis developed in 1993 have led to a significant advance in the production of high-quality size-tunable, monodisperse nanocrystals with high photoluminescence quantum efficiency (Grim *et al.*, 2015). This new material class has received substantial attention ever since, with an increasing range of applications, including photoactive compounds in nanobiohybrids.

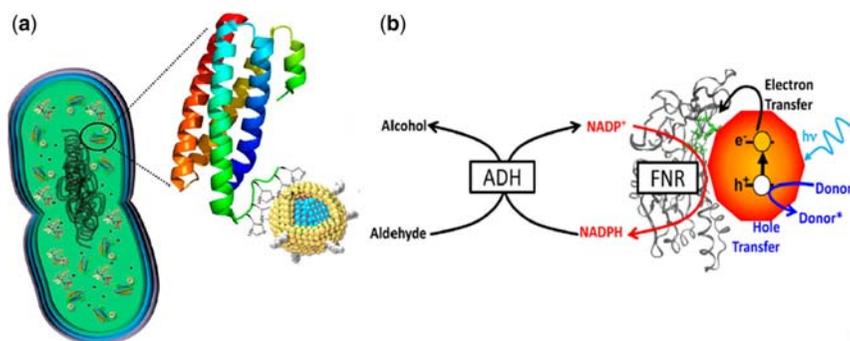
#### 12.5.4.2 Photosensitized nanohybrids

Quantum-dot bacteria nanobiohybrids combine the best of two worlds: the light-harvesting capabilities of semiconductors with the catalytic power of biology. Several quantum-dot based nanobiohybrids have been constructed, either with enzymes or microbial cells as the biological part of the hybrid. Electron transfer from illuminated quantum-dots to enzymes has been shown to support enzyme activity in hybrids of CdS with nitrogenase (Brown *et al.*, 2016a), CdSe with NADP<sup>+</sup> reductase (Brown *et al.*, 2016b) and CdTe with hydrogenase (Brown *et al.*, 2010). Sakimoto *et al.* (2016) showed the self-photosensitization of the non-photosynthetic bacterium *Moorella thermoacetica* by CdS bioprecipitation, and the photosynthetic production of acetate from CO<sub>2</sub> by this nanobiohybrid (Figure 12.10). Ding *et al.* (2019) further showed that CdS/ZnS core-shell quantum-dots penetrate *Azotobacter vinelandii* and *Cupriavidus necator* cells, where they bind to, respectively, the histi-tagged MoFe nitrogenase and FeS clusters of hydrogenases and quinones (Figure 12.11). Upon illumination, these nanobiohybrids demonstrated conversion of high yields of target products (ethylene and polyhydroxybutyrate) from CO<sub>2</sub>, comparable or even exceeding (>150%) native production levels (glucose as substrate).

Recently, nanobiohybrids using other inorganic light harvesters have been developed. Guo *et al.* (2018) evidenced that yeast (*Saccharomyces cerevisiae*) cells functionalized with indium phosphide (InP) quantum-dots harvested



**Figure 12.10** Left: Schematic presentation of the photosensitization of biohybrid systems, which combines the strengths of inorganic materials and biological catalysts by exploiting semiconductor broadband light absorption to capture solar energy and subsequently transform it into valuable  $CO_2$ -derived chemicals using the metabolic pathways in living organisms. Right: Overview of different nano bio-hybrid architectures. Utilizing electrons derived from a semiconductor light harvester (a), photosynthetic biohybrid systems channel reducing equivalents to generate  $H_2$  (b) to feed  $CO_2$  reducing microorganisms. These electrons may also go directly to the bacterium (c) to generate reduced  $CO_2$  products, such as acetic acid. This acetic acid may then be fed to genetically engineered organisms (d) to upgrade to a wide range of products (from Sakimoto *et al.*, 2017, and references therein).



**Figure 12.11** Schematic presentation of (a) the binding of a Cd/ZnS core shell quantum-dot to intracellular enzymes via histi-tag bonding upon incubating *Azotobacter vinelandii* cells in medium containing the quantum-dots (after Ding *et al.*, 2019) and (b) electron transfer in biohybrids of CdSe quantum-dots and ferredoxin NADP<sup>+</sup> reductase for photocatalytic regeneration of NADPH (After Brown *et al.*, 2016a, b). ADH: alcohol dehydrogenase; NADP<sup>+</sup>: nicotinamide adenine dinucleotide phosphate; FNR: ferredoxin NADP<sup>+</sup>-reductase.

photogenerated electrons from the illuminated nanoparticles and used them for the cytosolic regeneration of redox cofactors. Xu *et al.* (2019) reported on bioplastic production with light energy by coupling *Ralstonia eutropha* with the photocatalyst graphitic carbon nitride. Also intracellular gold nanoclusters can be used to photosensitize bacteria for solar fuel production (Zhang *et al.*, 2018).

#### 12.5.4.3 Photobioreactors for photosensitized nanobiohybrids

Light based production of fuels and fine chemicals using quantum-dot bacteria nanobiohybrids can make use of the vast experience of growing microalgae. Alternatively, solar fuels and biocommodities can be produced by growing the photosensitized nanobiohybrids in cell suspension bioreactors (Lens *et al.*, 2003). Several commonly used reactor systems for microalgae growth, including raceway ponds (Karya *et al.*, 2013), flat plate reactors (Rada-Ariza *et al.*, 2019), photo-active granules (Bing *et al.*, 2018) and algal biofilms (Moreno Osorio *et al.*, 2019) can be used. Microalgal culture systems have low biomass concentrations and problems with biomass/liquid separation, which constitute the main drawbacks in the scale-up and industrial application of microalgal processes (Gupta *et al.*, 2016). Microalgal biofilm systems could provide an alternative approach to resolve the microalgae-based biomass production challenges (Moreno Osorio *et al.*, 2019), and can thus also be explored for photosynthetic biohybrid systems. Biofilm photobioreactors can be grouped into three categories: permanently submerged, intermittently submerged and perfused systems (Berner *et al.*, 2015). However, photobioreactors that are used for algae axenic biomass cultivation do not always show equal results in wastewater treatment (Han *et al.*, 2017). There is thus a need to determine the efficiency and performance of photobioreactors when using quantum-dot bacteria nanobiohybrids for H<sub>2</sub> production and CO<sub>2</sub> derived fuels.

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