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Microorganism mediated biosynthesis of metal chalcogenides; a powerful tool to transform toxic effluents into functional nanomaterials

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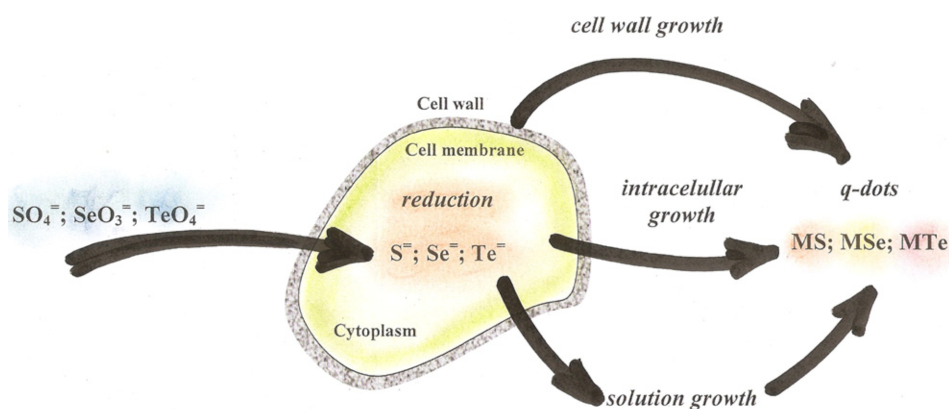
HIGHLIGHTS

- Removal of heavy metals by living matter is feasible trough biosorption and bioaccumulation
- Algae, fungi, bacteria and yeasts can synthesize CdS, CdSe and CdTe Q-dots
- Encapsulation of microorganisms in mineral gels provides building blocks for reactor design.
- Depletion of Cd with production of Q-dots can be achieved with modular bioreactors with entrapped cells

GRAPHICAL ABSTRACT



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ABSTRACT

Cadmium contained in soil and water can be taken up by certain crops and aquatic organisms and accumulate in the food-chain, thus removal of Cd from mining or industrial effluents – i.e. Ni-Cd batteries, electroplating, pigments, fertilizers – becomes mandatory for human health. In parallel, there is an increased interest in the production of luminescent Q-dots for applications in bioimaging, sensors and electronic devices, even the present synthesis methods are economic and environmentally costly. An alternative green pathway for producing Metal chalcogenides (MC: CdS, CdSe, CdTe) nanocrystals is based on the metabolic activity of living organisms. Intracellular and extracellular biosynthesis of can be achieved within a biomimetic approach feeding living organisms with Cd precursors providing new routes for combining bioremediation with green routes for producing MC nanoparticles. In this mini-review we present the state-of-the-art of biosynthesis of MC nanoparticles with a critical discussion of parameters involved and protocols. Few existing examples of scaling-up are also discussed. A modular reactor based on microorganisms entrapped in biocompatible mineral matrices – already proven for bioremediation of dissolved dyes – is proposed for combining both Cd-depletion and MC nanoparticle's production.

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1. Introduction

Industrialized civilization left the legacy of global-scale pollution of air, seas and land; this undesired side effect limits the present and future health of the environment as a whole. On recent decades civilization

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evolved developing concrete policies towards the recovery of the damaged environments and the incorporation of sustainable resources management. Nowadays, those main goals merge in novel approaches for remediation and waste treatment that convert undesired pollution agents in valuable products. To make real this two-fold win scenario, the mandatory chemical transformations involved in the process must be green and economically feasible. Biologically driven process can satisfy those requirements as they offer exquisite biochemical pathways, both in terms of product specificity and yield. One exciting example of this approach lies in the microorganism mediated formation of functional nanoparticles from heavy metal loaded effluents. In contrast with other molecular pollutants that can be totally biodegraded, heavy metals can only be treated in terms of separation through chemical transformation, as for example reduction to metal, precipitation forming insoluble less toxic solid phases, such as carbonates, phosphates or sulfides.

Physico-chemical methods for remediation of waste contaminated with heavy metals present several disadvantages due to the high requirement of reagents, most of them with high negative environmental impact. In this scenario the use of plants, plant extracts or microorganisms, such as bacteria, fungi, yeasts and algae to treat toxic pollutants is envisaged as an affordable technology inherently biocompatible (Mittal et al., 2013; Malik, 2004; Boopathy, 2000; Gadd, 2010). Since microorganisms cannot decompose heavy metals, detoxification strategies are based on the bioavailability minimization. One of those strategies is biosorption, or the ability of microorganisms to reversibly bind heavy metal ions at the cell surface. The functional groups present in the cell wall of algae, fungi and bacteria include carboxyl, amine, phosphonate and hydroxyl groups which play an important role in metal complexation (Volesky and Holan, 1995; Rangabhashiyam et al., 2014). In most cases metal biosorption follow a Langmuir or Freundlich isotherm and a pseudo second order kinetics, being independent on the cell metabolism (Srivastava et al., 2015; Febrianto et al., 2009). This enables the use of dead cells and cell fragments – i.e. bacterial S-layers (Allievi et al., 2011) – with the advantage of low cost procedures while it doesn't retain metabolic activity, thus being independent of the effluents toxicity and nutrient supply. A more challenging approach for remediation is bioaccumulation, or the uptake of metal ions by metabolically active organisms. Within this scheme heavy metals can be biosorbed by living organisms (passive uptake) and enter into the cell through the cell metabolic cycle (active uptake) (Malik, 2004). Therefore, bioaccumulation in living microorganisms opens the gate for complex and eventually relevant chemical transformations.

The specificity of metal uptake is enhanced when particular functional groups are present at the cell surface, such as siderophores chelates that reduce Fe^{3+} into Fe^{2+} being actively transported inside a bacterial cell (Neilands, 1995) or metallothioneins from eukaryotic cells synthesized under heavy metal stress which can complex Cu^{2+}, Zn^{2+}, or Cd^{2+} (Nies, 1992). Once at the surface, metal ions can remain bound to the biomolecules on the cell wall or they can suffer active transport towards the cytoplasm where they can be transformed to less harmful compounds or just accumulated in cellular vacuoles. Microorganisms present several specific and non-specific pathways to chelate, methylate, reduce or oxidize ionic compounds. Among others, Sulfate Reducing Bacteria (SRB) (Muyzer and Stams, 2008) are able to use sulfate as electron acceptor, producing sulfide. Other metalloid oxyanions, such as SeO_3^{2-} and TeO_3^{2-} can be also reduced to insoluble Se^0 and Te^0, respectively (Chung et al., 2006; Kim et al., 2013; Rajwade and Paknikar, 2003; Fellowes et al., 2013).

In the presence of metallic ions, the formation of sulfide, selenide or telluride leads to precipitation of the corresponding metal chalcogenides opening doors for new green routes for the obtainment of valuable nanoparticles, such as Q-dots. The discovery of magnetotactic bacteria was a milestone; (Blakemore, 1975) these bacteria orientate towards a magnetic field thanks to the intracellular synthesis of monodisperse magnetite (Fe_3O_4) or greigite (Fe_3S_4) nanocrystals within the magnetosome (Bazylinski and Frankel, 2004). The well-documented biosynthesis of nanocrystalline functional materials triggered biomimetic and bioinspired attempts to achieve different goals, among the materials science community. Biosynthesis of metal nanoparticles is easily conducted by several organisms, including plants, bacteria, fungi and algae due to the production of reductant species in many metabolic processes. It is well demonstrated that noble metal ions interact with carboxylates and amino groups at the cell wall, and these anchored ions further reduce developing nanoparticles (Beveridge and Murray, 1980; Klaus et al., 1999; Shedbalkar et al., 2014; Hulkoti and Taranath, 2014; Faramarzi and Sadighi, 2013). Spherical or polyhedral silver or gold nanoparticles have been obtained by several organisms including plants (Iravani, 2011; Narayanan and Sakthivel, 2010; Singh et al., 2015; Gericke and Pinches, 2006), revealing the capacity of living organisms for producing reductant, such as polysaccharides, as well as stabilizing species that inhibit of direct growth in particular directions. Even the mechanism of biosynthesis is not fully understood, there is a wide library of microorganisms producing metallic nanoparticles due to their interesting applications that range from biomedical applications to catalysis, drug delivery and biosensors. Silver nanoparticles are known to exhibit antimicrobial activity (Schröfel et al., 2014; Thomas et al., 2014; Mageswari et al., 2015; Suresh et al., 2010; Okafor et al., 2013), gold nanoparticles are widely used as biosensors (Daniel and Astruc, 2004) and palladium and platinum nanoparticles are used as catalysts in several industrial processes (Cheong et al., 2010; Arenz et al., 2005). The bioreduction of platinum and palladium has been less exploited, even it was demonstrated that Cyanobacteria *Calothrix* and *Leptololyngbya* produce reduction of Pt and Pd by nitrogenases (Brayner et al., 2007), and that algae *Chlorella vulgaris* reduces Pd(IV) by species produced during photosynthesis (Eroglu et al., 2013).

Microorganism-based bioremediation with conversion of toxic heavy metals to nanoparticles is an exciting approach that requires optimization. Biosorption by non-viable biomass is limited for the biosynthesis of oxides and chalcogenides because the metabolism is shut down; even it is not affected by the toxicity of the pollutants present in the effluents. Isolation and selection of heavy metal-resistant microorganisms is a critical issue that can be overcome by selecting strains tolerant to metal pollutants isolated from contaminated soils and waters. Chromosomal or plasmid genes are involved in the mechanisms of metal resistance rendering feasible genetic manipulation for strain improvement (Oger et al., 2003). Once selected, it is essential to determine if the interactions with model metal precursors lead to the formation of the wanted inorganic nanocrystals, and to evaluate that cells maintain a long term removal and nanoparticle biosynthesis ability. This analysis should take into account the optimization of microorganism's growth conditions, such as nutrients, pH, ionic strength and temperature in order to understand the metabolic pathways involved in heavy metal resistance and nanocrystal biosynthesis.

Though the synthesized nanoparticles will be biocompatible for the microorganism producing them, for bioremediation purposes the priority will be to deplete the concentration of toxic cations. Conversion of pollutants into valuable products is an important industrial and environmental challenge. In the particular case of nanoparticles, products require monodispersity and well defined shape, size and crystallinity (Koole et al., 2014). The separation and recovery of nanoparticles is not a minor issue, and for this task it is important to determine whether the nanoparticles were produced inside or outside the cells. Under the stress of being in contact with toxic media, living organisms may exhibit different responses. In an ideal scenario the knowledge of the biosynthesis mechanism will allow a better understanding of the cell functioning and also to know which are the variables that can be tuned to enhance the biosynthesis.

The biosynthesis of metal chalcogenides nanoparticles mediated by microorganisms was less explored than their metallic counterparts; even it can be a useful method for combining detoxification with green chemistry synthesis (Li et al., 2011; Durán and Seabra, 2012;

Jacob et al., 2016; Zhou et al., 2015). A critical analysis and perspectives of achieving this double task is still missing, therefore this mini-review summarizes the existing information with a discussion on protocols and on the possibilities of scaling-up and bioreactors design for technological purposes.

2. Metal chalcogenide (MC) nanoparticles

MC nanoparticles, in particular CdS, CdSe and CdTe are physically considered quantum dots (Q-dots) when their radii is lower than the Bohr exciton, i.e. <10 nm. These semiconductors with inherent functionality due to outstanding size dependent absorption and emission of visible light can be suitable for developing cutting edge technologies including optical devices (optical storage, light-emitting diodes), solar energy conversion or signaling of in vivo process as fluorescent labels (Bruchez et al., 1998; Jamieson et al., 2007).

The synthesis of a wide range of QDs was developed decades ago (Rossetti and Brus, 1982; Fojtik et al., 1984) using inorganic salts as precursors in aqueous-based reactions. Due to the presence of air and water polydispersed materials with relatively poor optical and crystalline properties were obtained by this route. Size control and monodisperse Q-dots were successfully achieved by an organometallic/organic-based synthesis using an inert atmosphere, appropriate precursors with origins in vapour deposition and coordinating solvents suitable for high-temperature reactions (Murray et al., 1993). This synthesis involves drastic conditions and toxic reagents such as the solvents (octadecene, trioctylphosphine (TOP)) and stabilizers that control growth, such as trioctylphosphine oxide (TOPO) (Peng and Peng, 2001). In addition, the obtained NPs are not biocompatible, being necessary the exchange of the capping molecules to achieve water solubility. Several attempts to synthesize these particles using softer conditions are being made, i.e. in aqueous solution, but monodispersion is still a challenge due to the difficulties of controlling nucleation and growth for highly insoluble compounds (Lesnyak et al., 2013).

Biosynthesis of CdS nanocrystals by microorganisms incubated with Cd(II) is mediated by short chelating peptides containing cysteine. The role of peptides is to provide a source of sulfur and to control the nucleation and growth of CdS nanocrystals (Dameron et al., 1989a, 1989b). Biosynthesis of CdSe or CdTe requires the external supply of both anion and cation's precursors to develop the reaction. Then, the employed microorganism has to be resistant to both moieties while being capable to simultaneously transform them into MC. In addition, a proper protocol is needed in order to avoid direct precipitation of insoluble chalcogenides.

Location of bio-synthetic processes is still a matter of debate. For a living organism the anion-cation encounter may be at the cytoplasm (intracellular growth) or in the external media (extracellular growth). It is accepted that reduction of oxochalcogenides (SO_4^{2-}, SeO_3^{2-}, TeO_4^{2-}) takes place at the cytoplasm by specific reductase enzymes (Keller et al., 2014; Ridley et al., 2006). Cations attached to the cell wall by electrostatic interactions are also excellent points for heterogeneous nucleation giving rise of particle growth. Furthermore, under stress as that induced by toxic heavy metals cells can expel different molecules that may enhance or inhibit the growth of MC nanoparticles. It should be noted that nanoparticles formed in the extracellular medium can migrate across the cell wall, usually by endocytosis, to the cytoplasm. Moreover, disruption of the cellular wall liberates to the external media the cytoplasmic components, giving a new pathway for extracellular growth.

Table 1 summarizes several biosynthesis of MC nanoparticles mediated by microorganisms as bacteria, fungi, yeast or algae. Most reports are focused on metal sulfides due to the natural abundance of sulfur in living organisms and its intrinsic low toxicity. The source of sulfide can be exogenous (e.g. reduction of SO_4^{2-} by enzymatic process) or endogenous (e.g. thiol groups belonging to proteins). Unfortunately, most studies are qualitative and yield data for conversion of dissolved Cd to MC nanocrystals is still missing for in vivo production of quantum dots.

Several microorganisms can synthesize different metallic chalcogenides, being good candidates for the production of mixed chalcogenides. For example, fungus *Fusarium oxysporum* incubated with the correspondent metal sulfate can synthesize by an enzymatic process at the external medium metal sulfide nanoparticles, such as PbS, ZnS, NiS, MnS and CdS with different morphology (Senapati et al., 2014; Ahmad et al., 2002; Dhillon et al., 2012).

Some strains of *Escherichia coli* synthesize CdS by culturing in a medium with sequential addition of $CdCl_2$ and Na_2S (Sweeney et al., 2004). 2–5 nm CdS wurtzite nanocrystals were obtained, and from cadmium mapping the cells by Energy-dispersive X-ray spectroscopy (EDS) it was concluded that nanocrystals growth is intracellular. However, with the added concentrations of $CdCl_2$ and Na_2S (1 mM each) CdS growth by direct precipitation reaction cannot be neglected, and the question remains open if intracellular cadmium is a consequence of intracellular synthesis or migration of nanoparticles produced in the external medium to the cytoplasm. It is worthy to note that under the same conditions CdS is not detected for other *E. coli* strains. An interesting mechanism has been proposed for the growth of CdS nanocrystals when the yeast *Schizosaccharomyces pombe* is cultured with $CdCl_2$ (Kowshik et al., 2002a). The mechanism involves phytochelatins (PC), a short peptide capable of binding heavy metals (Dameron and Winge, 1990; Clemens and Simm, 2003) whose production is enhanced in the presence of Cd^{2+}. A PC-Cd complex is formed and actively transported to a vacuole and once delivered in the cytoplasm the CdS nanoparticles are formed. A similar mechanism was proposed for the synthesis of PbS by *Torulopsis* sp (Kowshik et al., 2002a).

Incubation of the white rot fungus *Phanerochaete chrysosporium* with $Cd(NO_3)_2$ and thioacetamyde (TAA) as sulfur source results in the presence of CdS nanocrystals deposited onto the cell surface. Under the stress caused by Cd^{2+}, different biomolecules holding thiol groups (HS-R-COOH) may be secreted by the fungus and chelate the ion to decrease the toxicity (Cd-S-R). Gradually the hydrolysis of TAA releases S^{2-} that reacts with the free cadmium to form CdS nuclei. The complex Cd-S-R covalently binds to the CdS producing CdS nanoparticles which are capped with polypeptides (Chen et al., 2014). Genetically modified *E. coli* biosynthesize CdTe nanoparticles when incubated with $CdCl_2$ and K_2TeO_3 (Monrás et al., 2012). In this case it was demonstrated that glutathione (GSH) favors the biosynthesis, as the GSH acts both as TeO_3^{2-} to Te^{2-} intracellular reducing and capping agent. The procedure was successfully extended to the production of CdS nanocrystals by five strains of oxidative stress resistant bacteria collected in Antartic soils (*Pseudomona* spp) (Gallardo et al., 2014). For these strains sulfide production enhance CdS biosynthesis, but there is no relationship between CdS production and cellular thiol content. Highly luminescent CdS Q-dots were produced by an intrinsically high resistance to heavy metals bacteria (*Stenotrophomonas maltophilia*) incubated in cadmium acetate in the presence of L-cysteine as a sulfur source and capping (Yang et al., 2015). This engineered strain allows control of the size and distribution breadth with the incubation time; as growth time increases the mean diameter, standard deviation and quantum yield increase. This control provides a low cost, green synthesis of monodisperse controlled size MC nanocrystals.

Another interesting biosynthetic approach for combined remediation and MC production from Cd contaminated soils is the use of earthworms that produce Q-dots probably as a protective mechanism (Kominkova et al., 2014). *Lumbricus rubellus* exposed for 11 days to standard soil spiked with $CdCl_2$ and Na_2TeO_3 were shown to transport the precursors, via metallated metallothionein complexes, to the chloragogenous tissue located at the coelomic surfaces of the worm's gut, where they reacted to form Q-dots (Stürzenbaum et al., 2013). The authors propose a mechanism involving reduction of tellurite by glutathione reductase, and the production of H_2Te by reaction with

Table 1
Summary of microorganism mediated synthesis of MC.

	Organism	Compound	Synthesis	Ref.
Bacteria	*Shewanella oneidensis*	Ag_2S	Extracellular	Suresh et al. (2011)
	Escherichia coli	CdTe	Intracellular	Monrás et al. (2012), Kominkova et al. (2014)
	Escherichia coli	CdTe	Extracellular	Bao et al. (2010)
	Clostridiaceae sp	MnS	Extracellular	Liu et al. (2015)
	Escherichia coli	CdS	Intracellular	Monrás et al. (2012), Kang et al. (2008)
	Rhodopseudomonas palustris	CdS	Intracellular	Bai et al. (2009)
	Stenotrophomonas maltophilia	CdS	Extracellular	Zhou et al. (2015)
	Pseudomona spp	CdS	Intracellular	Gallardo et al. (2014)
Fungi	*Helminthosporum solani*	CdSe	Extracellular	Suresh (2014)
	Phanerochaete chrysosporium	CdS	Extracellular	Chen et al. (2014)
	Fusarium oxysporum	PbS, ZnS, MnS, NiS, CdS	Extracellular	Senapati et al. (2014), Ahmad et al. (2002)
Algae	*Chlamydomonas reinhardtii*	CdS		Hu et al. (2001)
Yeast	*Schizosaccharomyces pombe*	CdS	Intracellular	Kowshik et al. (2002a)
	Torulopsis sp	PbS	Intracellular	Kowshik et al. (2002b)
	Rhodosporidium diobovatum	PbS		Seshadri et al. (2011)
	Schizosaccharomyces pombe	CdS	Intracellular-extracellular	Dameron et al. (1989a)

NADPH. However, it should be noted that the worm's gut contains bacteria that may trigger the synthesis of nanocrystals.

3. Towards robust protocols

The mechanisms of microorganisms mediated synthesis of nanoparticles are not yet well understood, being these biosynthetic pathways a sort of "black boxes" that claims for special attention. In the following section a comprehensive list of mandatory recommendations regarding the obtainment of univocal experimental evidence is compiled, taking into account both literature reports, as well as our own experience.

It is important to diagram experiments in the frame of robust protocols, including the appropriate controls to univocally define the role of chosen microorganism in the nanoparticles biosynthesis. In this sense, we think that when planning an experiment of biosynthesis, certain precautions should be taken into account.

3.1. Toxicity and viability

Toxicity and viability assays must be done to determine the maximum concentration of the precursors that the microorganisms can resist without losing viability. It's important to note that this concentration may vary if the culture grows in a solid or a liquid media, since the diffusive and/or convective transport process of the precursors through the media is dramatically different. Thus, the bioavailability will be different in each case.

3.2. Cell density

Cell density must be defined in order to properly compare the inherent activity of different MO or different growth or physiological status of a given MO. The cell surface to precursor concentration ratio is a key parameter; since it defines both sorption and transport driving force it must be controlled and properly scaled in the experiment and controls.

3.3. Controls

The biosynthesis can be held with the microorganisms in the culture media or with them suspended in water or another solution. In both cases, a control of the synthesis without microorganisms has to be done to be sure that there is no nanoparticle production by the culture solution. Also, a control of the synthesis using the supernatant after harvesting the pellet is useful to see if the synthesis is due to different biomolecules secreted by the microorganisms during their growth. A suspension of non-viable or metabolically inactive microorganism could warn of passive biosynthesis driven by certain cell components that are active beyond metabolism.

It is possible that the biosynthesis is due to the enzymatic machinery of the microorganism "switched on" by the presence of the Cd^{2+} and chalcogenide precursors. In this case, the microorganism must be alive and viable to be able to produce the nanoparticles. On the other hand, different biomolecules secreted by the organism to the extracellular media or biomolecules of the cell wall may be the responsible of the synthesis. The microorganism not necessarily has to be viable, being enough for the synthesis to use an extract (obtained by filtration of the culture).

3.4. Growth phase

Another aspect that has to be carefully considered is the microorganism's growth phase that is usually divided in four steps: 1) lag phase: adaptation time to the new environment; 2) exponential phase: a period characterized by cell doubling; 3) stationary phase: the rate of growth is equal to the rate of dead and 4) decay phase: the culture is dying. The duration of each step is characteristic of each specimen. While the whole growth cycle of bacteria can take a couple of days, it can last weeks for yeasts or algae. The interaction of the nanoparticles precursors or the nanoparticles itself with the microorganisms can be different in each phase of growth. There are few reports comparing yields and properties of biosynthesized MC nanoparticles, even it has been demonstrated that for CdS synthesized by *Rhodopseudomonas palustris* the stationary phase gives higher yields (Bai et al., 2009; Sweeney et al., 2004).

4. Scaling-up

The eventual application of microorganism mediated synthesis of nanoparticles in bioremediation of toxic effluents requires scaling-up. Some attempts were reported for metallic nanoparticles. Ghorbani et al. (Ghorbani et al., 2011) designed a bioprocess to synthesize polydisperse Ag nanoparticles in a bench scale. Starting with a 15 L culture of *E. coli*, they filter it through polyvinylidene fluoride membranes. The filtrate then is mixed in a four part bioreactor with a solution of silver nitrate and the resultant colloid suspension is overflow to a reservoir. A more complex scaling-up was developed by Sanghi et al. (Sanghi and Verma, 2009) for the biosynthesis of CdS by the fungus *Coriolus versicolor*. The fungus immobilized in a glass column packed with tubular ceramic beads is able to grow and adhere to beads. After feeding, any external source of sulfur was removed before adding cadmium in order that CdS was produced by reaction of Cd^{2+} ions with thiolated proteins. A yellow precipitate composed by CdS particles of 100–200 nm diameters accumulated in the column. This method seems to be useful for both for bioremediating cadmium, but so larger nanoparticles are not useful for applications.

4.1. Bioreactor design

A prerequisite for any operational strategy for bioremediation with living organisms is their confinement in order to avoid dissemination of exogenous specimens in the environment, as well as to shield them from predators. The basic idea for a reactor design is to obtain macroscopic objects containing metabolically active cells that maintain long term viability (weeks, months) in order to build-up modular reactors retaining the biosynthesized nanoparticles whose components can be easily collected or replaced.

To be useful for the encapsulation of MC biosynthetic microorganisms, the encapsulating matrix need to fulfill some conditions: (i) biocompatible synthesis to ensure the survival of a significant fraction of microorganisms; (ii)the porosity of the network has to be tuned to allow the diffusion of nutrients and pollutants, but avoiding the escape of the produced nanoparticles; (iii)the matrix has to maintain its properties through changes in the operation conditions such as temperature, pH or humidity; (iv) the microorganisms have to remain biologically active. Known encapsulation matrices range from biopolymers (Muralidhar et al., 2001; Murua et al., 2008) to ceramics (Böttcher et al., 2004; Coradin and Livage, 2007; Coradin et al., 2009). Biopolymers such as alginate, quitosan and pectines are useful only for short-term operation for being biodegradable and macroporous. In contrast, inorganic oxide based gels results in robust mesoporous non-degradable materials, with adequate mechanical and chemical stability that are biologically inert and don't swell in aqueous or organic solvents. This concept has been successfully used for developing sensors (Ge et al., 2013; Liu et al., 2009), bioreactors (Avnir et al., 2006; Pressi et al., 2003; Lèonard et al., 2011) and bioremediation systems (Perullini et al., 2010; Raff et al., 2003).

Sol-gel chemistry provides a unique biocompatible pathway for encapsulation of living cells in inorganic oxide hosts giving true living materials (Livage, 2001; Nassif and Livage, 2011). The process is based on condensation of silicic acid or hydrolyzed alkoxydes giving a Si-O-Si network at room temperature. This living material can be used for the desired application as a soft but stift gel, or can be dried in biocompatible conditions for producing a solid with biological activity. Several strategies were developed in order to optimize the cell viability and stress due to deleterious effects of synthesis by-products (Perullini et al., 2008, 2011; Meunier et al., 2010). This general procedure schematized in Fig. 1.

Direct encapsulation of cells in inorganic hydrogels schematized in Fig. 1 leads to isolated cells that are not able to divide and proliferate inside the matrix unless undesirable fractures are formed. Cell division is possible in very dilute silica gels but, these matrices are too loosely bound for supporting mechanical stress (Eleftheriou et al., 2013). On the contrary, controlled drying of gels prepared from cells, precursors and ceramic powders or fibers in liquid N_2 produces a porous ceramic or Biocer with large pores giving enough space for cell division or germination of embedded spores (Soltmann and Böttcher, 2008).

The protection of cells by pre-encapsulation in fully biocompatible matrices before contact with silica sol avoids the direct contact of cells with silanols and by-products. This gives an efficient shield to the entrapped cells providing the sol-gel process is made in few minutes in order to minimize diffusion of cytotoxic species. The protected cells are further covered by a layer of silica, or immersed in a sol before gelation. The idea is to provide an optimal environment for the encapsulated cells by trapping in a biopolymer, while improving the transport and mechanical properties for long term viability within the mineral matrix. Encapsulation of cells in biocompatible polymers is mainly done with biopolymers that undergo cross-linking even in the presence of the cells (Hunt and Grover, 2010; Jen et al., 1996). Polysaccharides are the most used due to their low cost and proven biocompatibility with the encapsulated cells and the target (Nicodemus and Bryant, 2008), although proteins, such as collagen, gelatin or fibrin may also fulfill the requirements.

Calcium alginate is one of the most popular methods for cell encapsulation because it is easily formed upon contact between Na-alginate

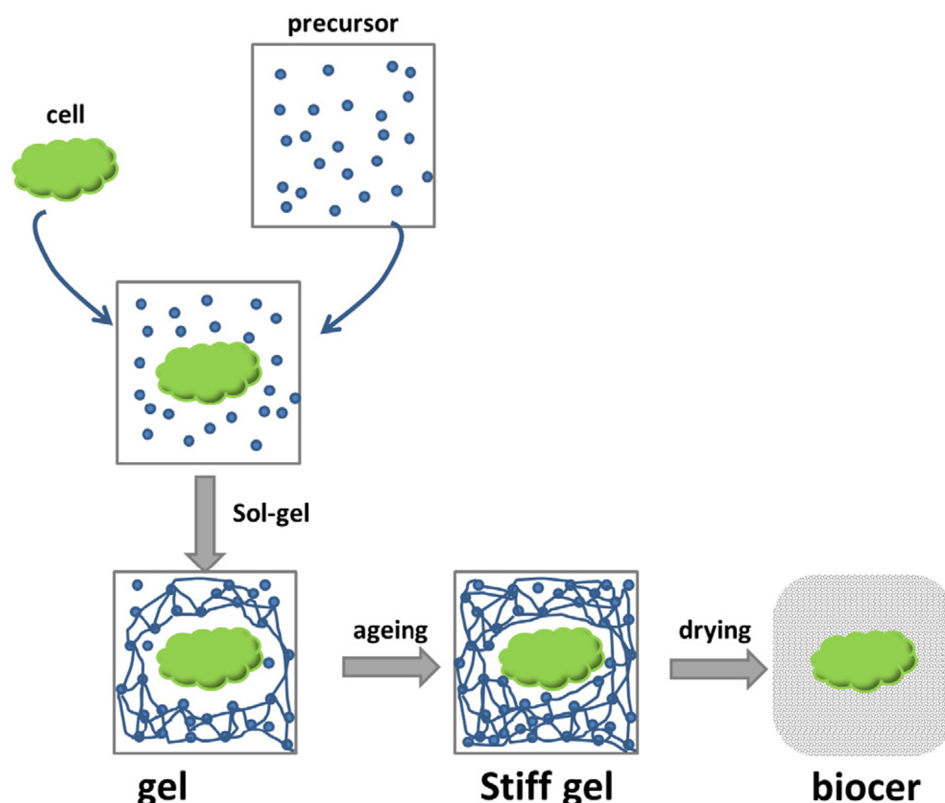

Fig. 1. General scheme of sol-gel encapsulation of cells. The precursor, oligomers or nanoparticles of hydroxylated SiO_2 forms a gel through condensation reactions. Drying of the gel results in a xerogel or, when cells are included, a biocer (Pompe et al., 2013).

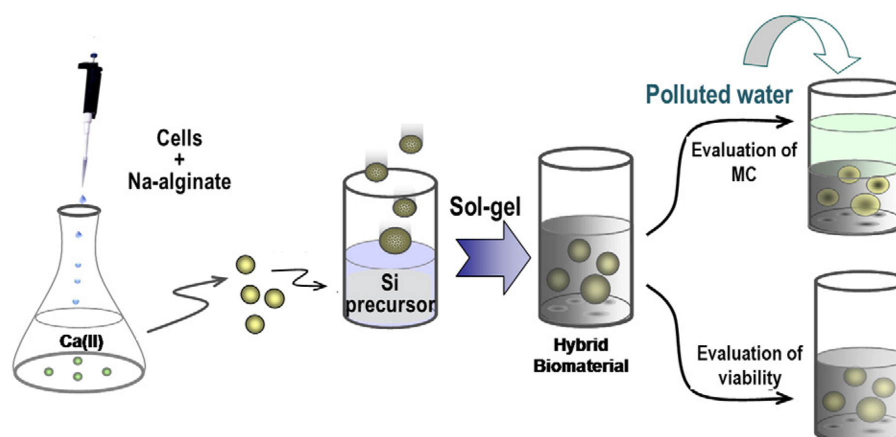

Fig. 2. Building living materials for bioremediation. Ca-alginate beads containing the selected microorganism are added to the silica precursors. Sol-gel should occur in few minutes. Once the gel consolidates viability of encapsulated moieties and production of CdS nanoparticles are monitored.

and Ca^{2+} aqueous solutions. This fast gelation kinetics allows to prepare beads of Ca-alginate by dropping the alginate solution in a $CaCl_2$ or $CaCO_3$ solutions. The size of the formed beads is a function of the drop, which in turn is given by the diameter of the tip employed for dropping, the Ca^{2+} concentration, the alginate concentration, the residence time of the bead in the Ca^{2+} solution, and the presence of other ions (Perullini et al., 2015). Entrapped microorganisms in calcium alginate networks turned out to be excellent biosorbents for metallic cations (Bang and Pazirandeh, 1999; Dash and Das, 2015).

The pre-encapsulation within alginate beads gives the possibility of macrocavities inside a mineral matrix where cells are able to duplicate and communicate, thus providing a long term living system producing both primary and secondary metabolites. With the sol-gel approach, the basic idea is to produce Ca-alginate capsules containing a specified strain -up ca 1 cm diameter- that are surrounded by a mineral matrix produced by any of the sol-gel pathways described above (Perullini et al., 2007, 2012). Within this approach schematized in Fig. 2 it is possible to build true living materials for specific purposes given by the encapsulated strain (Perullini et al., 2015).

The pores in the structure reduce the rate of diffusion of pollutants to the active cell. This translates in less toxicity for the microorganisms and controlled transformation of the pollutants into less harmful substances. The slower diffusion is related to adsorption at the host matrix (Perullini et al., 2014) which implies enhanced retention of Cd. This design was already used for degradation of dyes by encapsulating ligninolytic fungus *Stereum hirsutum* in silica hydrogels monoliths (Perullini et al., 2010). This bioreactor supports long term operation; the encapsulated mycelium tolerates higher levels of pollutant and exhibits more degradation capacity because of the constant production of enzymes involved in the process.

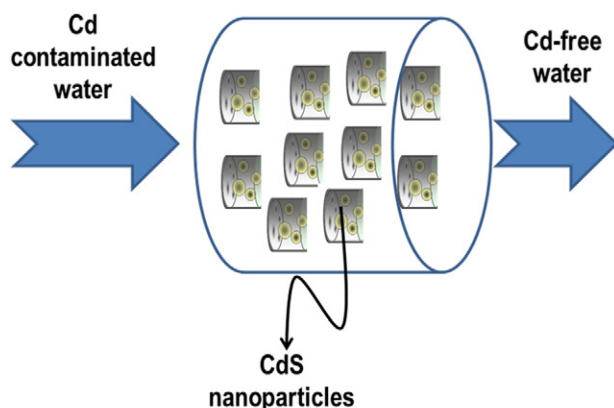

Fig. 3. Scheme of a bioremediation process build from modular bioreactors.

Besides organic pollutants, it has been reported that immobilized or encapsulated microorganisms exhibit higher resistance to metal ions than free cells (Soltmann et al., 2010). Biosynthesis of nanoparticles using encapsulated microorganisms has not been widely studied. To the best of our knowledge, there are no reports of biosynthesis of metal chalcogenides. Attempts of biosynthesis of gold nanoparticles by algae *Klebsormidium flaccidum* encapsulated in silica hydrogels and exposed to $HAuCl_4$ showed the green chloroplasts turned into purple, indicating the intracellular formation of gold nanoparticles (Dahoumane et al., 2012; Sicard et al., 2010).

The encapsulation of microorganisms in inorganic matrices is a promising platform for designing complex bioreactors, as schematized in Fig. 3. This strategy also provides materials with chemical and mechanical resistance for bioremediation with the extra outcome of producing useful compounds for nanotechnology. Biosynthesized nanoparticles will be easily recovered since they will be inside the mineral host and not widespread in the medium. The modular bioreactors also allow unlimited combination of selected microorganisms with sensitive metabolism for different pollutants.

5. Summary and perspectives

Biomimetic routes for producing Q-dots of metal chalcogenides provide also a way for environmental cleaning-up of highly toxic heavy metals, such as cadmium wastes of batteries and paints. Well-designed protocols would contribute to a better knowledge of mechanisms involved in the production of MC nanocrystals, thus giving a rationale for selecting biological species and incubation conditions in order to achieve a narrow distribution of MC nanoparticles with a predefined size. For technological purposes more efforts are needed in order to design bioreactors that should be based on encapsulated microorganisms for a safe biological manipulation and recovery of Q-dots.

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