# Chapter 7

# *In situ* and *ex situ* bioremediation of seleniferous soils and sediments

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## 7.1 INTRODUCTION

The importance of environmental selenium (Se) research has been increasingly recognized during the last decade (Nancharaiah & Lens, 2015a; Tan *et al.*, 2016). The concerns about Se toxicity began in the 1930s, when symptoms for alkali disease and blind staggers were observed in livestock grazing on grass grown on Se-enriched soil in South Dakota (Tinggi, 2003). On the other hand, Se deficiency was brought to the forefront in the 1960s with identification of a peculiar heart muscle disease symptom, called Keshan's disease, in China (Chen, 2012).

In animals and humans, selenium plays an important role in the redox regulation of intracellular signaling, redox homeostasis and thyroid hormone metabolism (Huawei, 2009; Papp *et al.*, 2007). To avoid deficiency and toxicity, the United States National Academy of Sciences Panel on Dietary Oxidants and Related Compounds recommended a dietary allowance of 55 µg Se $day^{-1}$ in humans and set an upper tolerable limit of 400 µg Se $day^{-1}$ (World Health Organization, 2011). The United Kingdom Expert Group on Vitamins and Minerals (2003) recommended a minimum intake of 60 µg Se $day^{-1}$ for women and 70 µg Se $day^{-1}$ for men.

The amount of selenium in the food chain, and thus in the human diet, depends on the selenium concentrations in the soil. Therefore, soil is the most important part of


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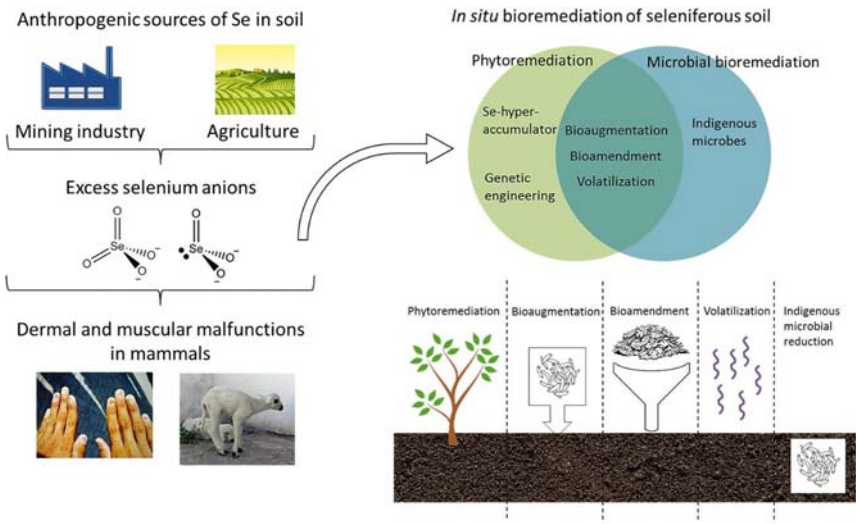


**Figure 7.1** General schematic of causes, effects and bioremediation options for seleniferous soils and sediments (Wadgaonkar, 2017).

the environment in selenium cycling (Hagarova *et al.*, 2005; Wadgaonkar *et al.*, 2018a). This chapter overviews research on the selenium cycle in the context of bioremediation of seleniferous soils and sediments. Figure 7.1 provides a general overview of seleniferous soil contamination and *in situ* bioremediation techniques applied. Recent studies on the natural and anthropogenic sources of selenium in soils and the adverse effects of elevated soil-Se content on the environment are overviewed in this chapter. Particular attention is paid to the cause and effect of selenium toxicity on flora and fauna associated with seleniferous soils and sediments as well as technologies for bioremediation of seleniferous soils coupled to selenium recovery.

## 7.2 METABOLIC ROLE OF SELENIUM

### 7.2.1 Selenium essentiality

In humans, selenium plays a complex metabolic role in protection of body tissues against oxidative stress, maintenance of the immune system and modulation of growth and development (Figure 7.2). Most of the assimilated selenium in tissues is available in the form of proteins called selenoproteins, in which selenium exists as selenocysteine (SeCys). Table 7.1 lists selenoproteins identified in humans and their functions. Selenium is an essential part of the antioxidant enzyme glutathione peroxidase which protects cell membranes from damage caused by lipid peroxidation. Selenoproteins like thioredoxin reductase and glutathione peroxidase play a role in cancer prevention by preventing intracellular oxidative

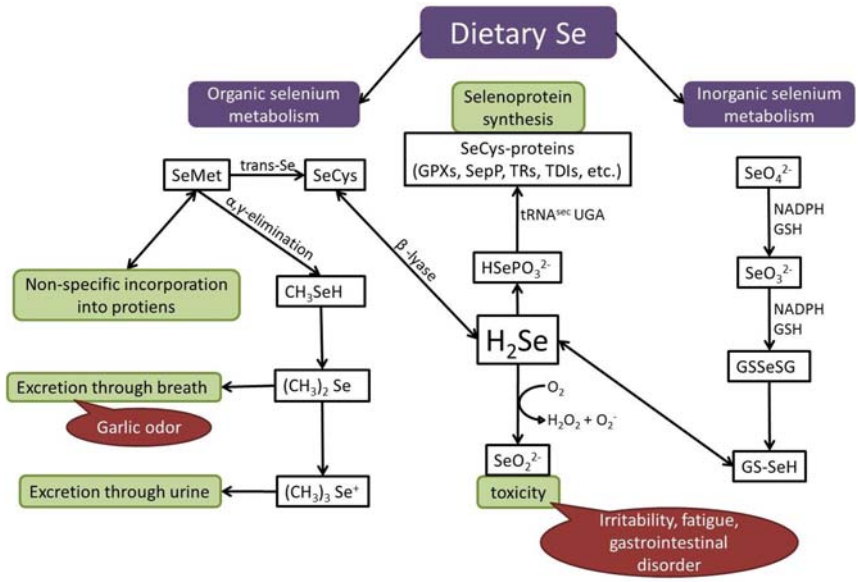


**Figure 7.2** Schematic representation of selenium metabolism in mammals. Se, selenium; SeMet, selenomethionine; SeCys, selenocysteine; $H_2Se$, hydrogen selenide; $HSePO_3^{2-}$, selenophosphate; $CH_3SeH$, methylselenol; $(CH_3)_2Se$, dimethyl selenide; $SeO_2$, selenium dioxide; $(CH_3)_3Se^+$, trimethyl selenonium ion; NADPH, nicotinamide adenine dinucleotide phosphate; GSH, glutathione; TRs, thioredoxin reductases; TDIs, thyroixine de-iodinases; GPXs, glutathione peroxidases; SepP, selenoprotein P (Fairweather-tait *et al.*, 2010; Huawei, 2009; Tinggi, 2003).

stress (Kalender *et al.*, 2013; Selenius *et al.*, 2010). Selenoproteins play an important role not only in the regulation of the intracellular redox state and anti-inflammatory functions, but may also play a significant role in glucose metabolism, calcium metabolism and glycoprotein folding (Rayman, 2012). Selenium forms a structural component of specific selenoproteins incorporated in the form of Se-methionine (Se-Met) in plants and Se-cysteine (SeCys) in animals. Active sites of selenoproteins consisting of SeCys have redox functions, such as scavenging free radicals (Figure 7.3), thus preventing oxidative stress and cancer (Misra *et al.*, 2015; Tapiero *et al.*, 2003).

## 7.2.2 Selenium toxicity

Selenium is a contaminant of potential environmental concern and is hence an important element from an environmental pollution point of view. The element was discovered to be toxic when the livestock grazing on grass grown in

**Table 7.1** List of selenoproteins identified in humans and their functions

| Selenoprotein | Tissue/Position | Role/ Functions | References |
|---|---|---|---|
| Glutathione peroxidase (GPX1) | Cell cytosol | Antioxidant reducing $H_2O_2$ and phospholipase A2 cleaved lipid hydroperoxides and storage vehicle for Se | Brown and Arthur (2001), Diamond (2015), Papp *et al.* (2007), Rayman (2012) |
| Glutathione peroxidase (GPX2) | Gastrointestine | Protection from toxicity of ingested lipid hydroperoxides in mammals; intracellular defense mechanisms against oxidative damage by preventing production of reactive oxygen species in colon | Brown and Arthur (2001), Papp *et al.* (2007), Rayman (2012) |
| Glutathione peroxidase (GPX4) | Membrane associated phospholipid hydroperoxide | Reductive destruction of lipid hydroperoxides and small soluble hydroperoxides; capable of metabolizing cholesterol and cholesterol ester hydroperoxides in oxidized low density lipoprotein | Brown and Arthur (2001), Diamond (2015), Papp *et al.* (2007), Rayman (2012) |
| Glutathione peroxidase (GPX3) | Extracellular | Possible antioxidant in renal tubules | Brown and Arthur (2001), Papp *et al.* (2007), Rayman (2012) |
| Selenium-binding protein 1 (SBP1) | Nucleus and cytoplasm of prostrate tissue | Toxification/detoxification process, cell-growth regulation, intra-Golgi protein transport, aging and lipid metabolisms | Diamond (2015), Papp *et al.* (2007) |
| Thioredoxin reductases-1 (TR1) | Cell cytosol | Regulation of intracellular redox state | Papp *et al.* (2007), Rayman (2012) |
| Thioredoxin reductases-2 (TR2) | Mitochondria | Regulation of intracellular redox state | Papp *et al.* (2007), Rayman (2012) |
| Thioredoxin reductases-3 (TR3) | Testis | Regulation of intracellular redox state | Papp *et al.* (2007), Rayman (2012) |
| Iodothyronine deiodinases type1 (DI1) | Kidney, liver, thyroid, brown adipose tissue | Inactive thyroxine metabolism to active 3,3'-5'triiodothyronine | Papp *et al.* (2007), Rayman (2012) |

| | | | |
|---|---|---|---|
| Iodothyronine deiodinases type2 (DI2) | Thyroid, central nervous system, pituitary, skeletal muscle, adipose tissue | Activation of thyroid hormones | Papp *et al.* (2007), Rayman (2012) |
| Iodothyronine deiodinases type3 (DI3) | Placenta, central nervous system, fetus | Inactivation of thyroid hormone | Papp *et al.* (2007), Rayman (2012) |
| Selenoprotein-P (SelP) | Plasma, brain, liver and testis | Selenium homeostasis; antioxidant activity | Brown and Arthur (2001), Papp *et al.* (2007), Rayman (2012) |
| Selenoprotein-W (SelW) | Brain, colon, heart, skeletal muscle, prostrate | Antioxidant activity, cardiac and skeletal muscle metabolism | Brown and Arthur (2001), Papp *et al.* (2007); |
| Selenoprotein-N (SelN) | Endoplasmic reticulum | Unknown | Papp *et al.* (2007) |
| Selenoprotein-S (SelS) | Endoplasmic reticulum | Role in innate immune response, inflammation, regulation of cytokines | Papp *et al.* (2007) |
| Selenoprotein-K (SelK) | Endoplasmic reticulum | Unknown | Papp *et al.* (2007) |
| Selenoprotein-R (SelR) | Cytosol and nucleus | Methionine metabolism, protein repair, antioxidant activity | Papp *et al.* (2007) |
| Selenoprotein-H (SelH) | Nucleus | DNA binding protein, regulation of glutathione synthesis genes and phase II detoxification | Kurokawa and Berry (2013), Fairweather-tait *et al.* (2010) |
| Selenoprotein-M (SelM) | Moderate expression on heart, lungs, kidney, uterus and placenta; and high expression in thyroid and brain | Unknown | Papp *et al.* (2007) |
| Seleno-phosphate synthetase | Cell cytosol | This enzyme regulates selenocysteine incorporation in selenoproteins to prevent toxicity | Papp *et al.* (2007) |

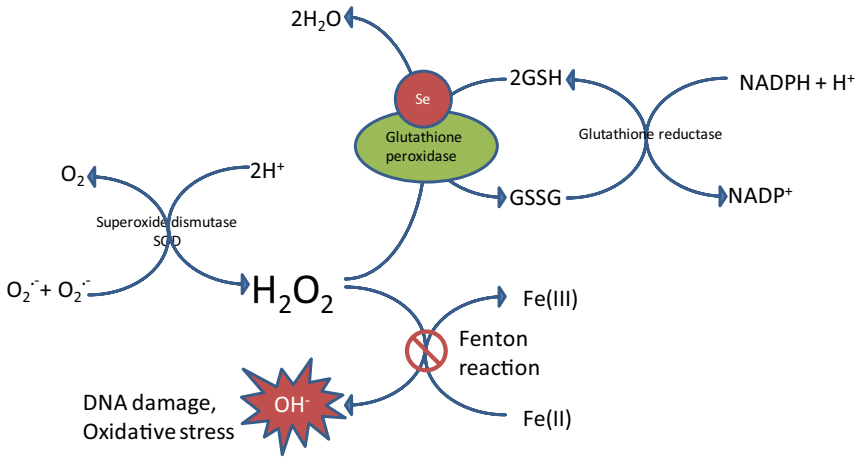


**Figure 7.3** Role of selenoprotein glutathione peroxidase in scavenging free radicals (Navarro-Alarcon & Cabrera-Vique, 2008).

selenium-enriched soil developed disorders known as alkali disease and blind staggers in 1930 in South Dakota (Tinggi, 2003). Selenium has a tendency to bioaccumulate in the aquatic environment and becomes toxic to aquatic organisms, such as fish, at elevated concentrations. Selenium becomes toxic to cormorants and other birds that prey on aquatic organisms harboring elevated levels of this element (Miller *et al.*, 2013). In 1984, high incidences of deformities and mortalities were recorded in waterfowl in the Kesterson wildlife reservoir (California, USA), where agricultural drainage water and industrial effluent containing a high selenium content was discharged.

Selenium bioaccumulation has exposed the fish and waterfowl in wetlands and evaporation ponds to a severe threat, which has caused deformities, impaired reproduction and eventually death of fish and birds (Zhang *et al.*, 2006). Transfer of selenium to the higher trophic levels and accumulation in the food chain due to selenium bioaccumulation (De La Riva *et al.*, 2014) caused additional damaging effects such as nausea, vomiting and diarrhea due to selenium poisoning (selenosis) in humans and animals along with dermal and neurological dysfunction associated with deformation of nails and hoofs, unsteady gait or even paralysis and cardiovascular symptoms (Duntas & Benvenga, 2014). Dietary uptake higher than 400 μg $day^{-1}$ was linked to hair and nail loss and disruption of the nervous and digestive systems in humans and animals (Misra *et al.*, 2015). Lemly (2014) calculated the monetary loss due to reduced fish productivity and studied the impact of selenium pollution in water bodies associated with high selenium concentrations on tissues of aquatic organisms (e.g., fish), particularly morphological abnormalities and teratogenic deformities.

### 7.2.3 Selenium deficiency

The perspective of researchers towards selenium changed in 1960, with the identification of a peculiar heart muscle disease symptom called Keshan's disease in selenium deficient populations in China (Chen, 2012). A selenium concentration in staple food lower than the critical standard of 100 μg $kg^{-1}$ can lead to the development of selenium deficiency diseases (Wang *et al.*, 2016). Selenium deficiency causes reproductive disorders and heart failure in humans, white muscle disease in young animals, fatal diseases such as hepatosis and Mulberry heart disease in pigs and exudative diathesis in poultry (Mehdi *et al.*, 2013). High incidences of white muscle disease and heart necrosis were observed in selenium deficient sheep and cattle in New Zealand and Western Oregon, USA (Tinggi, 2003). These and other studies have contributed to the understanding of physiological functions of selenium in higher animals and humans. The narrow window of 40–400 μg $day^{-1}$ between selenium deficiency and toxicity has led to selenium being appropriately termed as an 'essential toxin' (Lenz & Lens, 2009) and makes environmental selenium research (Winkel *et al.*, 2012) critical to maintain a balance between providing the necessary level and avoiding toxicity.

### 7.2.4 Selenium bioavailability

Both these Se toxicity and deficiency disorders are the ramifications of the Se bioavailability in the respective soils. Several studies are being carried out to resolve the issue of Se imbalance across the globe, where efforts are being made to remove Se from the seleniferous regions using *in situ* and *ex situ* bioremediation technologies (this Chapter; Bañuelos & Lin, 2005; Lindblom *et al.*, 2014) or fortify Se-deficient soils with organic and inorganic Se compounds (Chapter 9; Bañuelos *et al.*, 2015; Lyons, 2010).

## 7.3 SELENIUM GEOCHEMISTRY IN SELENIFEROUS SOILS AND SEDIMENTS

The selenium content in soil varies greatly throughout the world. Higher amounts of bioavailable forms of selenium in soils greatly influence the amount of selenium in the food chain. Elevated amounts of water-soluble selenium in soil can lead to contamination of water bodies and groundwaters due to the leaching caused by rainfall and irrigation (Wu, 2004). Effluent discharges from mining industries and coal fired power plants have resulted in large-scale Se deposition in marine sediments (Ellwood *et al.*, 2016). However, quantification of the total selenium does not actually give information about the chemical species, thus giving no information about its bioavailability to plants.

Various forms of selenium are soluble, exchangeable, bound to organic matter, sulfides, carbonates and oxides. Selenium occurs mainly in four oxidation states in soil, viz. selenate (VI), selenite (IV), elemental selenium (0) and selenide (-II).

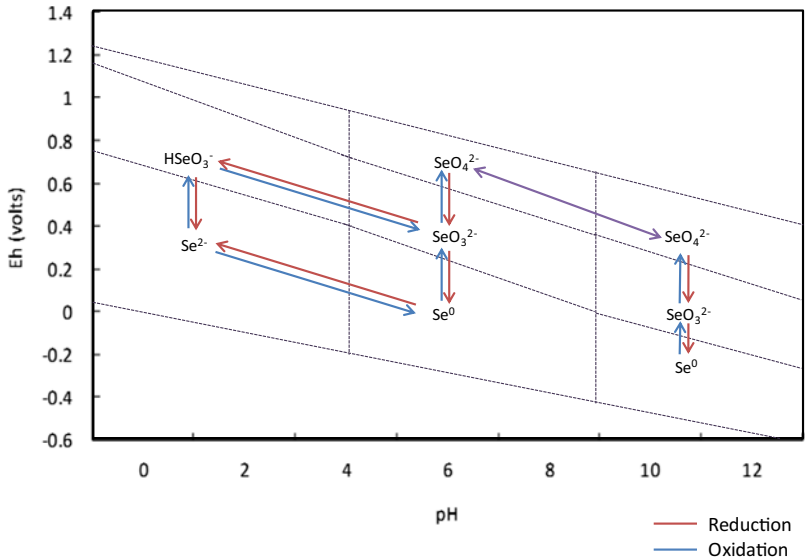


**Figure 7.4** Effect of pH and redox potential ($E_h$) of soil on Se speciation (Mayland *et al.*, 1989).

The chemical forms of selenium and their solubility in soil mainly depend on the prevailing redox conditions and pH (Figure 7.4), salinity and carbonate content of the soil (Hagarova *et al.*, 2005). Other factors that contribute to selenium speciation, and thus adsorption, bioavailability and toxicity, are organic matter content and composition, iron oxide levels as well as amount and type of clay (Table 7.2; Bajaj *et al.*, 2011; Schilling *et al.*, 2015).

In acidic clay soils and soils with a high organic matter content, selenium is mainly present in the form of selenides and selenium sulfides ($SeS_2/Se_nS_{8-n}$), which have limited solubility and bioavailability (Kabata-Pendias & Pendias, 2001). Selenium binds to proteins, fulvic acids, clay and organic matter in acidic soils (Cuvardic, 2003; Wu, 2004). In well drained neutral soils, selenium is predominantly available in the form of selenite (Mayland *et al.*, 1989). In alkaline and aerated soils, selenium occurs as selenate which is mobile and available to plants. Selenite is oxidized to selenate, the more mobile and bioavailable form of selenium in soil environments (Dhillon & Dhillon, 2014).

Se in seleniferous soil is often of lithogenic origin. For the soils in northwest India, selenium was transported via Se-rich sediments by seasonal rivulets from sub-Himalayan ranges called the Shiwalik hills in the north of Punjab, India (Dhillon & Dhillon, 2009). Intensive irrigation on the local agricultural lands during the last few decades has increased the Se deposition in the soil and has led to accumulation of up to 1.4 kg Se per hectare every year (Dhillon & Dhillon,

**Table 7.2** Soil conditions that affect the speciation, mobility and bioavailability of selenium (Kabata-Pendias & Pendias, 2001).

| Soil factor | Variables | Major Se form | Mobility |
|---|---|---|---|
| pH | Alkaline | Selenates | High |
| | Neutral | Selenites | Moderate |
| | Acidic | Selenides | Low |
| Redox potential | High | Selenites | High |
| | Low | Selenides | Low |
| Organic matter | Undecayed | Adsorbed | Low |
| | Decayed | Complexed | High |
| | Enhanced biomethylation | Volatilized | High |
| Clay content | High | Adsorbed | Low |
| | Low | Soluble | High |

2003), which affects agricultural crop yields (Figure 7.5). In order to avoid further contamination of these agricultural soils via rivulets containing Se-rich sediments, storage and sedimentation of the irrigation water may be practiced prior to irrigation on the agricultural fields (Dhillon & Dhillon, 2014). The Se-rich sediments may then be extracted and treated using a suitable technique. Se is a scarce and critical element (Nancharaiah *et al.*, 2016a) and its recovery from soil may not only assist in the clean-up of seleniferous soil, but the recovered Se may complement as a raw material for its wide range of applications from healthcare to electronic industries.

**Figure 7.5** Agricultural soil with high Se content, where wheat has been grown, in northwest India adversely affects the productivity of the crops and leads to bioaccumulation and biotransfer of Se to higher trophic levels (Wadgaonkar, 2017).

## 7.4 BIOREMEDIATION OF SELENIFEROUS SOILS

### 7.4.1 *In situ* treatment

Wadgaonkar *et al.* (2019a) set up microcosms to evaluate the effect of the organic amendment and bioaugmentation on reduction of Se in a seleniferous soil from northwest India. The organic amendment and bioaugmentation exhibited a similar Se removal performance as those of the control set-up without any amendment or bioaugmentation. This suggested that under ideal environmental conditions, the soil indigenous microbial population and organic content available in the soil were sufficient to achieve Se reduction *in situ*. Flury *et al.* (1997) also did not find major differences between the control and tests in a field study that attempted volatilization of Se from the Kesterson reservoir (California, USA). The drawback of the *in situ* method is that it only converts the bioavailable Se into insoluble Se forms, where the risk of its re-oxidation to soluble Se forms in soils with high redox potential and alkaline pH cannot be ruled out.

### 7.4.2 *Ex situ* treatment by soil flushing

Wadgaonkar *et al.* (2018b) characterized the physico-chemical parameters, total Se content and sequential extraction of Se in the soil collected from the seleniferous regions of Punjab (India). These characteristics allowed the further design of experiments for soil flushing and washing. Soil flushing was performed to assess Se migration in a soil column by simulating artificial rainfall or irrigation. Se migration and accumulation from the upper to the lower layers in the soil columns was observed suggesting reduction of soluble Se to insoluble Se forms in the deeper soil layers. Nevertheless, with time, this insoluble Se fraction may be slowly re-oxidized to soluble Se forms *in situ* and further contaminate groundwater.

### 7.4.3 *Ex situ* treatment by soil washing

Wadgaonkar *et al.* (2018b) optimized the soil washing of seleniferous soil using different washing solutions in order to achieve a maximum removal of bioavailable forms of Se from the seleniferous soil. Oxidizing agents were found to be most efficient in extracting Se from the studied seleniferous soil (Wadgaonkar *et al.*, 2018b). Biological treatment of the Se-rich soil leachate was further evaluated using microbial reduction in a continuous bioreactor (Wadgaonkar *et al.*, 2019a) and by phytoremediation using aquatic plants (Ohlbaum *et al.*, 2018). Figure 7.6 shows a schematic approach of the washing of seleniferous soil and biotreatment of the Se-rich soil leachate.

Prior to reconstitution of washed soil, suitability of the soil to be discharged in the environment should be determined. Unlike soil flushing, continuous agitation during soil washing disturbs the layers present in the soil column naturally, which might affect the soil characteristics. In addition, after soil washing, the soil characteristics and the bioavailability of Se might change in the course of time

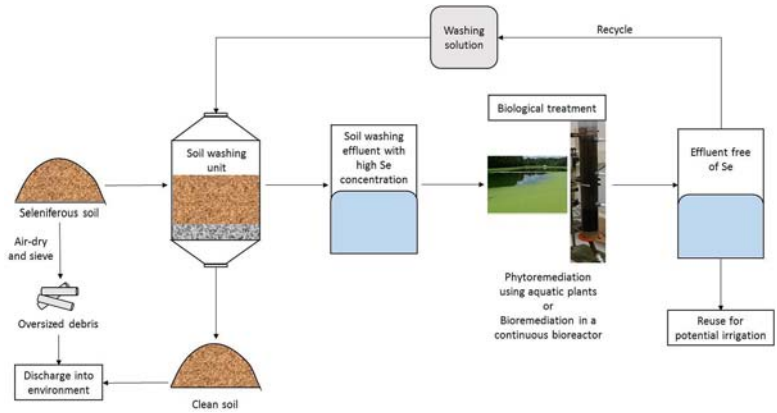


**Figure 7.6** Schematic approach of the washing of seleniferous soils and biotreatment of the Se-rich soil washing effluent (Wadgaonkar, 2017).

depending on the soil environmental conditions. Long-term experiments to determine the physico-chemical characteristics of the washed soil, as well as alterations in the Se fractions during sequential extraction must be performed. Fertility of the soil may be checked using germination tests. Pot experiments to compare plant growth and Se uptake in seleniferous soil and washed seleniferous soil may be performed.

Phytoremediation of Se-rich leachate containing residual oxidizing agents ($K_2S_2O_8$ and $KMnO_4$) was evaluated using aquatic plants such as *Lemna minor* and *Eregia densa* (Ohlbaum *et al.*, 2018). *L. minor* was found to be more efficient in removing Se from soil leachate and Se accumulation than *E. densa*. However, the presence of residues of the oxidizing agents in the Se-rich effluent adversely affected not only the Se removal efficiency but also growth of both plant species. Although application of oxidizing agents is promising for effective removal of Se from the seleniferous soils (Wadgaonkar *et al.*, 2018b), the use of chemical agents must be discouraged, as it might aggravate the post-treatment operations for both treated soil and the leachate.

## 7.5 BIOLOGICAL TREATMENT OF SELENIFEROUS SOIL WASHING WATER AND SELENIUM-CONTAMINATED GROUNDWATER

### 7.5.1 UASB reactors

#### *7.5.1.1 Treatment of soil leachate*

The leachate from seleniferous soils produced via *ex situ* treatment (soil flushing or soil washing) can be treated in an upflow anaerobic sludge bed (UASB)

reactor (see Chapter 6; Sinharoy & Lens, 2020). Se removal (up to 90%) and retention in the form of biogenic Se(0) in the granular sludge of the UASB was achieved during biological treatment of seleniferous soil washing water (Wadgaonkar *et al.*, 2019a). Along with the granular sludge, the indigenous soil microorganisms and the organic matter, extracted into the leachate during soil washing, play an important role to achieve efficient Se removal in a UASB reactor. Microbial reduction of soluble selenium oxyanions (selenate and selenite) to insoluble elemental selenium is the best available cost effective and eco-friendly option (Nancharaiah & Lens, 2015b).

Depending on the selenium concentration, external electron donors need to be dosed for complete selenate removal. Most of the research on bioreduction of Se oxyanions using anaerobic granular sludge utilize expensive carbon sources such as lactate (Dessì *et al.*, 2016; Mal *et al.*, 2016), methanol (Eregowda *et al.*, 2020) or glucose (Espinosa-Ortiz *et al.*, 2015). Few studies have explored the possibility of using inexpensive electron donors like methane (Lai *et al.*, 2016) as the sole electron donor for bioreduction of Se oxyanions. Wadgaonkar (2017) investigated the anaerobic bioreduction of selenate to elemental Se by marine lake sediment in the presence of methane as a sole electron donor. Complete bioreduction of selenate was observed in serum bottles under high pressure conditions and in a biotrickling filter (BTF) reactor. However, due to the slow growing nature of the inoculum (Bhattarai *et al.*, 2017), the operation time to achieve complete selenate removal from the medium was significantly higher than that of other studies that provide lactate as the electron donor (Dessì *et al.*, 2016; Mal *et al.*, 2016).

#### 7.5.1.2 Presence of tellurium

Anthropogenic activities such as mining and refinery industries can lead to contaminated soil-water environments containing both Se and Te (Perkins, 2011). Recovery of Se and Te as by-products from copper mining industries focusses on anode slime which contains high concentrations of Se and Te (Jorgenson, 2002). Both Se and Te are considered as critical elements because of their wide range of applications and increasing demand in the electronic industries (Nancharaiah *et al.*, 2016a; Ramos-Ruiz *et al.*, 2016).

Wadgaonkar *et al.* (2018c) investigated the effect of metalloid co-contaminants (Te) on Se removal and simultaneous removal of both Se and Te from wastewater. Simultaneous removal of both selenite and tellurite from synthetic wastewater by anaerobic granular sludge was sustainable in a lab-scale UASB reactor. Microbial transformations converted both oxyanions (selenite and tellurite) to their respective elemental forms (Se(0) and Te(0)), which were entrapped in the extracellular polymeric substances (EPS) matrix of the granular sludge, easing the recovery of these critical elements. Interestingly, characterization using high-end

microscopic and spectroscopic techniques revealed the formation of biogenic Se (0)-Te(0) nanostructures, entrapped in the sludge granules, during simultaneous reduction of Se and Te oxyanions (Wadgaonkar *et al.*, 2018c).

### *7.5.1.3 Presence of other oxyanions*

#### 7.5.1.3.1 Granular versus biofilm reactor systems

The practical application of biological treatment for Se-laden wastewater may have important limitations because of the presence of co-contaminants such as nitrate ($NO_3^-$) and sulfate ($SO_4^{2-}$) (Tan 2017) and heavy metal ions (e.g. $Ni^{2+}$) (Mal *et al.*, 2016). Tan *et al.* (2018d) demonstrated that a UASB reactor was capable of removing selenate ($SeO_4^{2-}$) in the presence of $NO_3^-$ and $SO_4^{2-}$ without prior inoculum adaptation, achieving a 100% $NO_3^-$, 30% $SO_4^{2-}$ and 80% Total Se ($Se_{tot}$) removal efficiency after 90 days of reactor operation. Comparing the effect of the oxyanions $SO_4^{2-}$ and $NO_3^-$, individually, on Se removal in continuous operation, a correlation with different reactor configurations, microbial growth system, and co-oxyanions was observed. $SO_4^{2-}$ was shown to be a controlling factor in biofilm systems using a drip flow reactor (DFR) (Tan *et al.*, 2018a) and biotrickling filter (BTF) (Tan *et al.*, 2018c) achieving both higher Se removal and more biofilm growth/formation when $SO_4^{2-}$ was present. In contrast, the UASB reactor did not reveal any changes, either increase or decrease, in Se removal efficiencies, when $SO_4^{2-}$ was removed from (Tan *et al.*, 2018d) or included (Tan *et al.*, 2018c) in the feed solution. In contrast to $SO_4^{2-}$, it was observed that the removal of $NO_3^-$ from the feed solution possibly caused an increase in $Se_{tot}$ concentration in the UASB effluent, thus negatively impacting Se removal efficiencies by the end of the reactor run (Tan *et al.*, 2018d).

The influence of $NO_3^-$ and $SO_4^{2-}$ on $SeO_4^{2-}$ removal is most likely interlinked with (i) the bioreactor type used, (ii) possible interaction among the bioreduced products of the oxyanions, and (iii) microbial community changes that occurred during the operation. $SeO_4^{2-}$ reduction can be carried out by various microorganisms including denitrifiers and sulfate reducers. The microbial community analysis revealed the presence of high relative abundances of denitrifiers along with a small proportion of sulfate reducers, irrespective of the presence of $NO_3^-$ (Tan *et al.*, 2018a, 2018d). Enzymes such as $SeO_4^{2-}$ reductase, $NO_3^-$ reductase or periplasmic $NO_2^-$ reductase have been reported to catalyze $SeO_4^{2-}$ reduction to $Se^0$ (DeMoll-Decker & Macy, 1993; Nancharaiah & Lens, 2015a). It was hypothesized that the presence of $NO_3^-$ can promote a higher state of metabolic activity in microorganisms (Oremland *et al.*, 1999). This implies that $NO_3^-$ plays a significant role in shaping the microbial community in the bioreactor and specific metabolic pathways/activities of denitrifiers could be linked to the increase of $SeO_4^{2-}$ removal in the presence of $NO_3^-$ and its by-products (Lai *et al.*, 2014).

#### 7.5.1.3.2 Adsorption coupled to biological selenium removal processes

Tan *et al.* (2018b) and Cálix *et al.* (2019) investigated the possibility of including an ion exchange (IX) process in the treatment scheme for removing both $SeO_4^{2-}$ and $SO_4^{2-}$ from synthetic mine wastewater. Tan *et al.* (2018b) validated the applicability of Amberlite® IRA-900, a strong anionic IX resin, for the simultaneous removal of $SeO_4^{2-}$ and $SO_4^{2-}$ achieving >70% adsorption efficiencies. Modified Langmuir multi-component isotherms using a complete competition model indicated that IRA-900 was not selective towards $SeO_4^{2-}$ and that both oxyanions competed for the active sites. The non-selectivity of IRA-900 gives the advantage of adsorbing both oxyanions saving the need for pre-treatment of $SO_4^{2-}$ to avoid inhibition of $SeO_4^{2-}$ adsorption. Chemical regeneration was optimal at 20 min using 0.5 M HCl and the resin was reusable for up to six adsorption-desorption cycles.

Despite the advantages of biological techniques, there are still issues with reaching the regulatory discharge limit for $Se_{tot}$. Cálix *et al.* (2019) evaluated the feasibility of combining two unit processes, an IX column and a UASB reactor, for the overall improvement of $SO_4^{2-}$ and $Se_{tot}$ removal (Figure 7.7). The IX process was evaluated as a pre- or post-treatment process for the biological process. IX as a post-treatment was demonstrated to be less effective due to competition with other bioreduced products and the presence of suspended solids. On the other hand, IX as a pre-treatment system allowed the UASB reactor to receive lower concentrations of $SO_4^{2-}$ and $SeO_4^{2-}$ facilitating a better biological removal process and achieving an overall higher removal efficiency of 99% $SO_4^{2-}$ and 97% $Se_{tot}$. The final treated effluent contained <100 mg $L^{-1}$

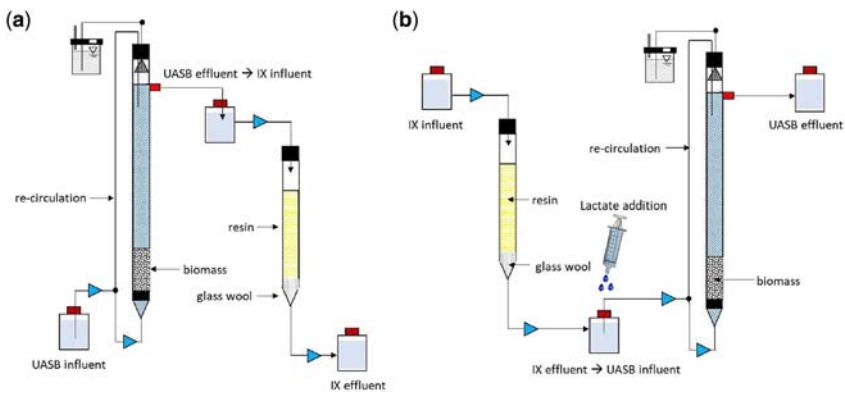


**Figure 7.7** Schematic of the integrated treatment system comprising of an IX column and a UASB reactor. The process flow configuration was evaluated as either (a) IX as post treatment of the UASB or (b) IX as pre-treatment of the UASB influent (Cálix *et al.*, 2019).

$SO_4^{2-}$ and <0.3 mg $L^{-1}$ $Se_{tot}$ (Cálix *et al.*, 2019). Though the $Se_{tot}$ concentration still does not reach the regulatory discharge limit, it should be noted that all dissolved Se was removed and only colloidal Se was remaining. Therefore, the addition of a (electro-)coagulation process (Staicu *et al.*, 2015b) as a finishing step could further lower the $Se_{tot}$ concentration and achieve the regulatory limit (Nancharaiah & Lens, 2015b). Additionally, with the lowering of the $SO_4^{2-}$ concentration supplied to the biological process, less COD requirement would be needed, decreasing the operational costs. Finally, bioregeneration of the resin by reduction of the adsorbed $SO_4^{2-}$ and $SeO_4^{2-}$ by biogranules was shown to be feasible and could potentially be used as an alternative to chemical regeneration. Cálix *et al.* (2019) demonstrated that integration of unit processes can allow the full removal of Se and $SO_4^{2-}$ from wastewaters.

#### 7.5.1.4 Presence of heavy metals

Tan *et al.* (2018c) demonstrated the importance of the chemical oxygen demand (COD)/$SO_4^{2-}$ ratio and the impact of $Ni^{2+}$ on $SeO_4^{2-}$ removal in a UASB reactor in comparison to that of a BTF reactor. Poor $SO_4^{2-}$ removal (<30%) was observed at ~1.8 COD/$SO_4^{2-}$; while increasing the COD/$SO_4^{2-}$ ratio to 2.8 improved the $SO_4^{2-}$ removal to >80% with generation of high sulfide ($HS^-$) concentrations (up to 250 mg S $L^{-1}$). Addition of $Ni^{2+}$ to these sulfidogenic conditions allowed simultaneous removal of Ni and sulfide via co-precipitation. Formation of $Ni_3S_2$ precipitates was confirmed by X-ray diffraction (XRD). However, $Ni^{2+}$ addition negatively impacted both $SeO_4^{2-}$ and $SO_4^{2-}$ removal by 20–50%, possibly due to the sudden metal toxicity to the biofilms/biogranules. Compared to the BTF, the UASB reactor performance recovered quickly. This could be attributed to the robustness of anaerobic sludge granules, allowing for better response to operational changes and stress.

### 7.5.2 Aerobic reactors

Selenium-containing wastewaters can be treated, aerobically, in activated sludge systems, where the selenium oxyanions are reduced to elemental selenium by the microorganisms present in the activated sludge flocs. Aerobic reduction of selenium oxyanions to either volatilized selenium compounds followed by gas trapping (Kagami *et al.*, 2013) or selenium trapped in the biomass of aerobic microorganisms such as *Bacillus cereus* (Dhanjal & Cameotra, 2010) and *Escherichia coli* (Dobias *et al.*, 2011) as well as biomass from aerobic granular sludge (Nancharaiah *et al.*, 2018), continuous (Jain *et al.*, 2016) or sequencing batch (Mal *et al.*, 2017) reactors, activated sludge provides a one-step process for treatment of selenite-containing wastewaters.

A continuously operated activated sludge reactor operated at neutral pH and 30°C removed 33.98 and 36.65 mg of total selenium per g of total suspended solids (TSS) at TSS concentrations of 1300 and 3000 mg $L^{-1}$, respectively (Jain *et al.*,

2016). The selenium fed activated sludge showed better settleability and hydrophilicity, but poorer dewaterability at higher TSS concentrations as compared to the control activated sludge. The selenium fed activated sludge also showed a less negative surface charge density as compared to the control activated sludge (Jain *et al.*, 2016). However, the operated activated sludge reactors crashed upon continuous feeding of selenium after 10–20 days at the applied loading rates and operation could not be recovered (Jain *et al.*, 2016), most likely due to irreversible toxicity of selenite to the aerobic bacteria. This selenite toxicity could be overcome by sequencing batch feeding of the activated sludge system (Mal *et al.*, 2017), which further allowed the integration of selenite removal with ammonium removal from the wastewater.

Some aerobic selenite reducing bacteria are capable of tolerating and reducing high selenite concentrations, for example, endophytes present in plants growing on seleniferous soils (*Pseudomonas moraviensis* subsp. stanleyae; Staicu *et al.*, 2015a) or as contaminant in minimal salt medium (*Delftia lacustris;* Wadgaonkar *et al.*, 2019b). *D. lacustris* not only reduces Se oxyanions to elemental selenium, but also produces seleno-ester compounds (organo-Se compound) depending on the initial concentrations of Se oxyanions in the medium (Wadgaonkar *et al.*, 2019b). Chakraborty *et al.* (2019) grew this bacterium in a co-culture with *Phanerochaete chrysosporium* and immobilized the bacterium on the fungal pellets. Thus, the co-culture was able to couple the reduction of selenite to elemental Se(0) (by *D. lacustris*) to the degradation of phenol (by *P. chrysosporium*).

### 7.5.3 Membrane reactors

Membrane filtration technology using reverse osmosis (RO) and nanofiltration (NF) has been applied for treating Se-laden wastewaters (Chapter 6; Staicu *et al.*, 2017). However, despite the effectiveness of these technologies, the operational cost is high due to the required hydraulic pressure. Membrane bioreactors, on the other hand, combine the biological process and particle separation and are a cheaper option compared to NF or RO (Hu *et al.*, 2017). Although membrane technology still has the major drawback of higher operating cost than conventional processes, this can be overcome by decreasing the downstream unit processes and overall reduction of the reactor size (Hu & Stuckey, 2006).

Figure 7.8(b) suggests a possible reactor configuration for an anaerobic reactor with a submerged membrane. One of the main disadvantages of using membrane technology, particularly in an anaerobic system, is the higher potential for biofouling. This could, however, be mitigated by using a high flow rate and biogas recirculation as a means for membrane scouring. Additionally, it is possible to either fabricate membranes with covalent Se attached to the membrane or utilize the colloidal $Se^0$ as a means to reduce biofouling. Vercellino *et al.* (2013a, 2013b) reported a reduction of the biofilm formation and thickness by $>$ 5 logs, as well as

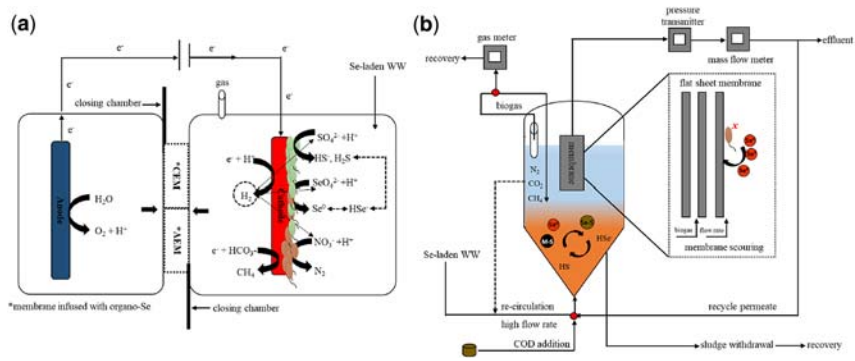


**Figure 7.8** Innovative reactor configurations proposed for Se-laden wastewaters with co-contaminants: (a) two-chamber bioelectrochemical system (BES) for autotrophic reduction of oxyanion utilizing hydrogen as electron donor generated electrochemically with an anionic/cationic exchange\membrane AEM/CEM) for ion transfer from one chamber to another and (b) modified upflow anaerobic sludge blanket reactor with submerged membrane module (e.g., flat sheet or hollow fiber membrane) utilizing biogas production and high flow rate for membrane scouring (Tan, 2017).

a flux loss decrease from 55 to 15% due to the biofouling attenuation when organo-Se was covalently attached to the membrane surface.

## 7.5.4 Bioelectrochemical processes

A bioelectrochemical system (BES) employs microorganisms and electrodes to catalyze redox reactions and this system can be utilized in a two-chamber microbial fuel cell using either the anode, cathode or both as bioelectrodes (Ntagia *et al.*, 2020; Sánchez *et al.*, 2020). Electroactive microorganisms can extract electrons that can be utilized to generate electricity, treat wastewater by redox reactions and also recover nutrients (i.e., P, N), sulfur (Ntagia *et al.*, 2020) and metals (Nancharaiah *et al.*, 2016a). Removal of individual selenate (Sravan *et al.*, 2020), selenite (Catal *et al.*, 2009), $NO_3^-$ (Nancharaiah *et al.*, 2016b) and $SO_4^{2-}$ (Luo *et al.*, 2017) using BESs has been reported in the literature. However, application of a BES to complex Se-laden wastewater with different co-contaminants present has yet to be reported.

Although lactate is an efficient electron donor and carbon source, alternative electron donors/carbon sources are recommended since using lactate adds operational cost considering that most industrial Se-containing wastewaters have a low COD content. Autotrophic reduction of $NO_3^-$, $SO_4^{2-}$ and $SeO_4^{2-}$ using $H_2$ as the electron donor was found to be successful in membrane biofilm reactors (Ontiveros-Valencia *et al.*, 2016; van Ginkel *et al.*, 2011). It is, therefore, feasible to carry out autotrophic reduction of $NO_3^-$, $SO_4^{2-}$ and $SeO_4^{2-}$ at the

biocathode side by autotrophs utilizing the generated $H_2$. Figure 7.8(a) suggests a possible reactor configuration that utilizes the ability of a BES to generate hydrogen gas ($H_2$) through an electrochemical process. An IX membrane (anionic or cationic) separates the two chambers and allows for charge balancing by moving $H^+$ and $OH^-$ as well as other possible value-added chemicals produced in each chamber. The IX membrane can be infused with organo-Se to reduce the biofouling that can occur at the side of the bioelectrode (Vercellino *et al.*, 2013b).

## 7.6 COUPLING SELENIFEROUS SOIL REMEDIATION TO RESOURCE RECOVERY

### 7.6.1 Biofortification

Extraction of selenium from seleniferous soil by soil washing and its accumulation in anaerobic granular sludge (Dessì *et al.*, 2016; Mal *et al.*, 2016) or aquatic plants (Li *et al.*, 2020a, 2020b; Ohlbaum *et al.*, 2018) helps with easier recovery of Se from soil. The selenium rich granular sludge or aquatic plants may be used for biofortification of selenium in crops (see Chapter 9). In addition, Se-containing aquatic plants can be considered for developing a dietary selenium supplement. Similar application of Se hyperaccumulator plants has been proposed to compensate the effects of selenium deficiency in selenium deficient regions (Moreno *et al.*, 2013; Yasin *et al.*, 2014). Several studies (Curtin *et al.*, 2006; Lyons, 2010) have investigated the effect of the addition of inorganic-Se (selenate and selenite) containing fertilizers for selenium biofortification in food crops in selenium deficient countries such as the UK and New Zealand. In Finland, selenium deficient soils have led to a low selenium status in humans and animals. A nationwide strategy was adopted on application of multimineral fertilizers on agricultural soils to improve selenium dietary intake (Parkman & Hultberg, 2002).

Maintaining accurate and low selenium dosage in agricultural land can be difficult owing to diverse cropping systems and selenium losses due to leaching and volatilization. Also, since selenium is a scarce resource with low recovery potential when applying foliar fertilizer, amendment with organic-Se such as Se-enriched hyperaccumulator plant material to Se-deficient soils may prove as a better fertilizer alternative. Bañuelos *et al.* (2015) concluded that amending soils with organic selenium sources such as biomass of the hyperaccumulator *S. pinnata* is useful for enriching food crops, such as broccoli and carrots, with organic-Se in selenium deficient regions of the world. Bañuelos and Mayland (2000) suggested that Canola (*Brassica napus*) grown as a selected plant species for field phytoremediation of Se-contaminated soils may be harvested and utilized as Se-enriched forage for marginally Se-deficient lambs and cows to help meet their normal selenium intake requirements.

In the case of biogranules, it is possible to simply dry the biomass and use it as a fertilizer for soil augmentation and foliar application for those regions with Se

deficiency to increase the Se bioavailability (Wang *et al.*, 2016). In the case of biofilms with carriers, detachment of biofilms through centrifugation/sonication or heating can be considered. However, it should be noted that the biomass produced while treating soil washing liquor or industrial mine wastewater also contains various other contaminants that can be harmful to the environment. Therefore, detailed studies on the toxic effect and health ramification of using Se-rich biomass as fertilizer should be conducted.

### 7.6.2 Recovery of biologically produced nanomaterials

Resource recovery is an important aspect to consider when applying biological treatment techniques. Recovered and purified $Se^0$ nanoparticles, along with metal and sulfur complexes, can be reused for various semiconductor (i.e., batteries) and catalyst applications, as an adsorbent for toxic metals (i.e., mercury), and as antimicrobial agents for medical devices and membranes (Chapter 10; Zonaro *et al.*, 2015). Biomass from the bioreactors contains various valuable materials such as metals, sulfur or sulfur complexes, and Se components that have the potential to be recovered (Sinharoy & Lens, 2020). The recovery process of Se and other complexes from biomass has, however, not yet been explored. So far, a method for recovery and purification of $Se^0$ nanoparticles from biomass was suggested as a series of solid-liquid steps: high-speed centrifugation, and sonication followed by hexane separation (Jain *et al.*, 2015). As such, an eco-friendlier and simple recovery approach should be developed.

Other components in the soil leachate may influence the production and composition of the biogenic Se nanostructures. Along with biogenic Se(0), the possibility of formation of chalcogenide alloys (e.g., Se-Te and Se-S) or metal chalcogenide nanoparticles (e.g., PbSe, CdSe and CuSe) in continuous bioreactor systems cannot be ruled out. The determination of the structure and composition of the biogenic selenium nanoparticles requires detailed analysis of the granular sludge, for example, using high-end techniques such as transmission electron microscopy coupled with energy-dispersive X-ray spectroscopy (TEM-EDS), X-ray photoelectron spectroscopy (XPS) and Raman spectroscopy. These analytical techniques are not routine and require specific equipment.

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