Selenium recovery from wastewater based on exclusively extracellular selenium nanoparticles production

Abstract

Most of the selenium contamination in the United States is primarily caused by the disposal of wastewater from coal-fired power plant, Flue Gas Desulfurization (FDG), and agricultural drainage. Selenium occurs in various forms, including selenate (SeO_4^{2-}), selenite (SeO_3^{2-}), selenide (Se^{2-}) and elemental selenium (Se^0). Common removal methods include physicochemical processes like anion exchange, reverse osmosis, and oxide sorption, along with microbial processes, particularly for the predominant selenate form. While physical processes like reverse osmosis are highly efficient, they do not detoxify selenium. In contrast, microbial processes offer a clean, non-toxic, and eco-friendly solution that enables the recovery of elemental selenium nanoparticles (SeNPs) both intracellularly and extracellularly. These microbial-synthesized SeNPs are more biocompatible and stable, with applications in photovoltaic cells, solar panels, radar equipment, the glass and ceramic industry, and mammographic detectors. However, conventional bioreactors typically produce intracellular Se^0, requiring significant energy to separate SeNPs from biomass. Therefore, the overall objective of this dissertation is to develop a sustainable and efficient methodology to exclusively produce extracellular SeNPs from selenium-laden wastewater using biocathodes of bioelectrochemical (BEC) reactors.

The production of intracellular SeNPs in biological water treatment processes poses a significant challenge for selenium recovery, as it requires energy-intensive steps to break down microbial cells and separate the SeNPs. The first objective of this study is to demonstrate that biocathodes can produce significantly more extracellular SeNPs compared to conventional bioreactors using transmission electron microscopy (TEM) and energy-dispersive X-ray spectroscopy (EDS). Analysis of the cathodic microbial community revealed that the relative abundance of *Azospira oryzae, Desulfovibrio, Stenotrophomonas,* and *Rhodocyclaceae* increased from less than 1% in the inoculum to 10% - 21% at steady state in the BEC reactor. These microorganisms are known to produce both intracellular and extracellular SeNPs in conventional bioreactors, but they preferentially produce extracellular SeNPs (97-99%) on the biocathode. This preference is likely due to cellular energetics: by producing extracellular SeNPs, microbes save energy by avoiding the need to transport SeO_4^{2-} and electrons from the cathode into the cells. The feasibility of extracellular SeNP production on the biocathode is supported by the presence of high concentrations of C-type cytochrome, known for its ability to transfer electrons from electrodes to microbial cells and facilitate the reduction of SeO_4^{2-} to SeNPs on the cell membrane.

Removal of SeO_4^{2-} from selenate-contaminated wastewater is challenging due to the commonly co-existing and competing anions of sulfate (SO_4^{2-}) and nitrate (NO_3^-). The second objective of this study investigated the reduction of SeO_4^{2-} to elemental selenium (Se^0) in a cathode-based BEC reactor and a conventional biofilm reactor (*i.e.*, an upflow anaerobic reactor) under various conditions. The simulated wastewater contained SeO_4^{2-} at a typical concentration of 5 mg Se/L, SO_4^{2-} at a typical concentration of 1,000 mg S/L, and NO_3^- at concentrations that varied from 0 to 10 mg N/L. The BEC reactor achieved 99% selenium removal, compared to 69% in the conventional reactor, which was hindered by greater sulfate reduction, leading to competition for electrons and significant selenide production. Selenide is usually assumed to be

minimal and therefore not measured in the literature. This simplification may significantly overestimate selenium removal when the influent sulfate concentration is very high. NO_3^- in the influent of the BEC reactor promoted selenium removal when it was less than 5.0 mg N/L but inhibited selenate removal when it was more than 7.5 mg N/L. This was supported by the microbial community analysis and intermediate (nitrite) analysis. The findings emphasize the need to optimize flow rates, retention times, and microbial inoculum to improve selenium reduction and recovery in wastewater treatment from sources like FGD and agricultural drainage.

Microbial processes are crucial for the redox transformations of toxic selenium oxyanions. The third objective of this study explored the mechanisms of SeO_4^{2-} reduction by the facultative anaerobe *Azospira sp.* A9D-23B using chemical kinetics and enzyme activity assays in both BEC and conventional bioreactors. The study found significantly different mechanisms of SeO_4^{2-} reduction to elemental Se^0 between the two reactor types. TEM coupled with EDS revealed that the biocathode in the BEC reactor produced significantly more extracellular Se^0 (99%) compared to the conventional bioreactor (65%), demonstrating the strain's preference for extracellular reduction on the biocathode. Further investigation into selenate reductase activity showed that enzymes associated with the outer membrane and periplasm in the BEC reactor exhibited higher selenate reduction activity (18.31 ± 3.8 µmol/mg-min) compared to those in the conventional bioreactor (3.24 ± 2.9 µmol/mg-min). Conversely, enzymes in the inner membrane and cytoplasm had lower activity in the BEC reactor (5.82 ± 2.2 µmol/mg-min) than in the conventional bioreactor (9.18 ± 1.6 µmol/mg-min). The high specific activity and lower Km (114 ± 3 µM) of the outer and periplasmic selenate reductases suggest that *Azospira sp.* A9D-23B is more effective for extracellular Se^0 recovery in BEC reactors. These findings are crucial for selecting bioreactors aimed at exclusive extracellular selenium recovery.

A biofilm model is a risk-free environment, that enhances accuracy through better visualization while saving time, money, and resources. Additionally, it can generate parameters to support the design of reactors at scale. Hence, the fourth objective of this study was to develop and parameterize a multispecies one dimensional biofilm model to simulate and understand the interactions between SeO_4^{2-} and SO_4^{2-} at the biocathode of a BEC reactor. The model accurately simulated all observed trends in the BEC reactor, with acetate, the electron donor, not being limited. In the cathode chamber, selenate reducing bacteria (SeRB) and selenate and sulfate reducing bacteria (SeSRB) were the dominant active biomass species, accumulating more than 35% at the cathode electrode and being responsible for SeO_4^{2-} reduction (64 - 94%), while SeSRB and sulfate reducing bacteria (SRB) species were responsible for SO_4^{2-} reduction (2 - 21%). However, the percentage abundance of SRB at the cathode electrode was low. At the anode chamber, acetate was used as an electron donor and carbon source by acetate oxidizing bacteria (AOB) (42%). The model further accurately predicted the influence of current density on the anode and cathode overpotentials, biofilm thickness, and the concentration of dissolved species in both chambers of the reactor. This is the first computational evaluation of the interaction between SeO_4^{2-} and SO_4^{2-} in a BEC reactor, providing deeper insight into the bioelectrochemical reaction mechanisms at the electrodes. It offers a convenient pathway to optimize operating conditions and improve the performance of the biocathode in BECs for the highest removal rate of selenate from industrial wastewater.