Chapter IV

Optimization of physico-cultural parameters for rapid synthesis of Selenium nanoparticles

Different cultural and physical parameter affects the microbial growth and their products. Current chapter describes optimization of different conditions including cultivation medium, temperature, pH, light intensity, concentration of Selenium dioxide (SeO$_2$), and time of reaction for bio-synthesis of Se NPs via *Pentoea agglomerence*.

Work presented in this chapter has been published in journal:

4.1. INTRODUCTION

Cornucopia applications are developed in nanotechnology by merging theories of biology, physics, chemistry and material science. An extra attention has been paid on the nanomaterials or nanoparticles (NPs) due to their unusual properties compared to their counterparts. The Se is essential trace element and plays an important role in microbes, animal and certain plants etc. In human it is the part of seventeen selenoprotein families. These selenoproteins have antioxidant properties and help in cellular processes like biosynthesis of dNTPs, in removing signaling peroxides, reduce oxidized proteins and membranes, regulating redox signaling, thyroid hormone metabolism, protein folding and Se transportation.

Recent studies have demonstrated that Se is cancer chemopreventive agent, which reduce the incidence of lung, colon, prostate and liver cancer. Few fungicides and antidandruff shampoos primarily are based on Se compounds. Although Se was majorly ignored as a therapeutic agent for over 100 years due to its toxicity, but in recent years it has been reported that Se NPs have lower toxicity so researchers are trying to understand it’s effect in biological systems. Industrial point of view, Se has applications in the field of electronics like xerography, solar cells, photography, and rectifiers as well as in removal of unwanted green tone in glass caused by iron oxide impurities. The Se NPs are highly reactive due to this they are being used for the synthesis of many other fundamental Se functional metal chalcogenides such as ZnSe, CdSe, Ag$_2$Se and SnSe etc.

From an environmental perspectives, Se NPs are used as catalysist in photodegradation of textile dyes and also in removing mercury from the gaseous phase. These findings have aroused interests of the scientific community in this complex element. That is why there is increase in demand for Se NPs in biomedicine, industry and environment to accomplish these demands, there is a critical need to increase their production yield. Physical and chemical method greatly accepted for the synthesis of Se NPs in short time. But both of these methods have few limitations like use of toxic chemicals are unavoidable which basically are impede their direct biomedical uses. These techniques are complicated, expensive, and resulting in production of...
harmful toxic waste, which are hazardous not only to the ecosystem but as well to human beings also\textsuperscript{14}.

Microbial cells are capable of synthesing Se NPs by aerobic and anaerobic modes. For a large scale production aerobic mode is mostly preferred due to the difficulties in maintenance of anaerobic condition at large scale. Large scale manufacturing of any material need to be optimized and a little contribution has been made previously on finding the effect of cultural and physical conditions on the biosynthesis of Se NPs\textsuperscript{16}. The synthesis of Se NPs at nano-range level with high yield is still a challenging and critical issue. In order to increase the yield and the shelf-life (stability) of Se NPs with minimum investment, it is essential to fix the cultural conditions along with various physic-chemical parameters like pH, light intensity, and temperature etc.
4.2. MATERIALS AND METHODS

A number of experiments were performed concerning the rate of synthesis and stability of Se NPs. The parameters such as medium, pH, temperature, light intensity, concentration of SeO₂ and time of reaction etc., were standardized for the rapid and maximum synthesis of Se NPs. For each condition, there was respective control. All experiments were performed in triplicate.

4.2.1. Effect of growth medium

Effect of six different media, namely, R2A medium\textsuperscript{15}, Tryptic soya broth (TSB)\textsuperscript{16}, DeMan-rogosa-sharpe broth (MRS)\textsuperscript{17}, Luria-Bertani broth (LB)\textsuperscript{18}, Liquid Modified Marine broth (LMM)\textsuperscript{19}, Nutrient broth (NB)\textsuperscript{20} were studied for the formation of Se NPs. All the media were screened for the optimum and stable NPs synthesis. The \textit{Pentoea agglomerence} were grown in 500 mL erlenmeyer flasks, each containing 100 mL of test medium and SeO₂ 9 mM. The synthesis of Se NPs was carried out using procedure mentioned in Chapter 3.

4.2.2. Effect of pH

To study the effect of pH on synthesis of Se NPs, \textit{Pentoea agglomerence} was grown in media having different pH values (3-10). The pH of the supernatant containing Se NPs was also maintained with 1N HCl and 1M NaOH.

4.2.3. Effect of temperature

To study the effect of different temperatures on synthesis of Se NPs, \textit{Pentoea agglomerence} were inoculated in medium containing SeO₂ and incubated for different temperatures i.e. 0, 20, 40, 60, 80, and 100°C for overnight.

4.2.4. Effect of light intensity

To study the effect of light intensities, \textit{Pentoea agglomerence} containing medium and SeO₂ was exposed to different light intesntities like 14.5 (15), UV - 94.1 141.9 (40), yellow fluorescence -189.9 Lux (40 W), sun-750 × 100 Lux light and dark. TES-1332 digital Lux meter was used for measuring the light intensity.
4.2.5. Effect of SeO$_2$ concentration

Substrate concentration plays an important role in amplification of the biosynthesized Se NPs. To optimize concentrations of SeO$_2$, enrichment broth with varying concentrations of SeO$_2$ (1 mM to 10 mM) were prepared and inoculated with Pentoea agglomerence. After incubation Se NPs synthesis was studied by measuring UV-Vis spectra.

4.2.6. Effect of reaction time

To study the effect of reaction time on the rate of synthesis of Se NPs, Pentoea agglomerence were inoculated in medium containing SeO$_2$ and incubated. After every 2 h, the reacting samples were withdrawn and UV-Vis spectrum was measured and water was used as blank or reference.
4.3. RESULTS AND DISCUSSION

4.3.1. Effect of growth medium

The Se NPs synthesized via bacterial cells were catalyzed by special types of reductase\textsuperscript{16,21}. Different types of media component were metabolized by the bacterial cells and secrete different metabolites and different kinds of proteins. Enzyme responsible for reduction of Se oxyions must be secreted in large quantity to get maximum production of Se NPs by corresponding bacterial cells. Bacterial cells (*Pentoea agglomerence*) grown on previously mentioned six different media to evaluate the effect of different media on synthesis of Se NPs. Microbial growth and formation of Se NPs (emergence of red color) were observed in TSB followed by MRS and then LB agar.

The maximum growth and Se NPs production was found in TSB which might promote the extracellular reductase secretion, wherein the bacterial growth without SeO\textsubscript{2} reduction was observed in R2A, NB and LMM medium. The SEM measurement of Se NPs synthesized by *Pentoea agglomerence* in media TSB, MRS and LB showed that Se NPs synthesized in TSB were relatively smaller in sizes (80-85 nm) while were larger particle sizes in MRS (100-300 nm) and LB broth (100-200nm) (detailes are given in Fig.4.1a, b and c).
Fig.4.1. The Se NPs obtained from: (a) TSB, (b) MRS and (c) LB broth.

Extra-cellularly secreted proteins capped Se NPs showed multiple effects on the dispersion, including potential screening of the surface charges that might help in maintaining the repulsion between the particles, or bridging type interactions this might be responsible for larger particle sizes in MRS and LB medium.

4.3.2. Effect of pH

The pH has significant role in synthesis of stable Se NPs. In alkaline (pH ~ 8-9) condition maximum synthesis of Se NPs were observed but after 38 days aggregation was dominant. Alkaline condition triggered the gene expression of 243 enzymes which could responsible for other cell functioning as well as Se NPs production also. Hydroxyl ions in large amount required for the reduction of metal ions that’s why there was enhanced synthesis observed at alkaline pH.

The Se NPs synthesized in acidic condition (pH ~ 4-5) confirmed aggregation type behaviour. At neutral pH, less but stable production of Se NPs was evidenced and until 45 days aggregates were not seen. Therefore, at pH ~7 and ~9 stable Se NPs were obtained and they
didn’t change to black amorphous form, thereby neutral pH was responsible for long shelf life and alkaline pH for maximum yield.

### 4.3.3. Effect of temperature

Temperature is a critical parameter in determining the rate of any chemical and biological reactions. Enzymes are sensitive to the change in temperature and green synthesis of Se NPs depends on enzyme, so it is crucial to determine optimum temperature for maximum synthesis of Se NPs.

The highest growth of *Pentoea agglomerence* was observed at 40-50°C and maximum extracellular protein secretion was seen at 60°C (Fig.4.2). Heat shock might responsible for high protein secretion. Special type of reductase required for synthesis of Se NPs at 50°C. That’s why absorbance peak and surface plasmon increased gradually up to 50°C after that it decreased (Fig.4.3).

![Graph showing effect of temperature on extracellular protein secretion by Pantoea agglomerans.](image)

**Fig.4.2.** Effect of temperature on extracellular protein secretion by *Pantoea agglomerans*.

Increase in the number of Se NPs and size of individual Se NPs contributed for increased absorbance. Though protein secretion by *Pentoea agglomerance* at 60°C was maximum but
maximum synthesis found at 50°C, some other proteins were secreted by *Pentoea agglomerence* which otherwise were not responsible for Se NPs production (Fig. 4.2b and 4.3b).

Fig. 4.3. Effect of temperature on optical absorbance of synthesized Se NPs.
Fig. 4.4. Effect of temperature on Se NPs synthesised by Pantoea agglomerans: (a) 30, (b) 40, (c) 50 and (d) 60°C.

The SEM image of Se NPs synthesized at 50°C revealed average particle size of 60 nm and amazingly up to 43 days there was no agglomeration. While Se NPs developed at 30, 40 and 60°C have particle sizes of 100, 100, 110-150 nm, respectively (Fig.4.4b). At 70-100°C, no growth of Pentoea agglomerence and there was no colour change indicating that incompatibility of this temperature range for fabrication of Se NPs.

4.3.4. Effect of light intensity

Photoinduced method is frequently used by the researchers for the synthesis of metal NPs and nanocomposite materials. In order to study the effect of light intensity, Pantoea agglomerans were exposed to various light intensities. Rapid extracellular synthesis (12 h) of Se NPs was carried out under Sun light this could be due to generation of free electrons of
photosensitization of some aromatic compounds, which could be used by Se oxyions for the synthesis of Se NPs. About 48 h was required under 189.9 and 141.9 Lux light intensities. Under 14.5 and 94.1 Lux intensities even after 72 h, there was no evidence for Se NPs synthesis. Little color change was evidenced in dark. From this observation it was concluded that high light intensity favored the rapid reduction of Se oxyions.

4.3.5 Effect of Se oxyion concentration

In an enzyme catalyzed reaction, substrate concentration is crucial issue to achieve maximum product yield. So to get maximum Se NPs, we optimized the substrate i.e. Se oxyion concentration as Se NPs synthesis is enzyme catalysed reaction\textsuperscript{16,26}. To optimize the substrate concentration, \textit{Pantoea agglomerans} were exposed for increasing concentrations of Se oxyions.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig4_5.png}
\caption{Effect of Se oxyion concentration on absorbance of synthesized Se NPs.}
\end{figure}

In the UV- Vis spectra, several peaks were seen, of which highest peak was for 9 mM concentration of Se oxyions (Fig.4.5). Due to toxicity effect on bacterial cells, higher
concentrations than 9 mM hamper the growth of *Pantoea agglomerans*thus synthesis of Se NPs were not evidenced.

4.3.6. Effect of reaction time

Reacting sample from culture flask at regular time interval (2 to 12 h) were removed and UV-Vis spectra were measured.

![Graph showing absorbance over time](image)

**Fig.4.6. Effect of reaction time on Se NPs synthesis.**

UV-Vis spectra showed continuous increase in absorbance at 593 nm and the reaction was completed in 12 h (Fig. 4.6). Initially, the color of the filtrate was pale-yellow and as time passes, colour changed to red followed by dark-red.
REFERENCES

G. Gonzalez-Gil, P.N.L. Lens and P.E. Saikaly, Front Microbiol. 7(2016) 571