# Optimization of *Serratia nematodiphila* using Response surface methodology to silver nanoparticles synthesis for aquatic pathogen control

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# Optimization of *Serratia nematodiphila* using Response surface methodology to silver nanoparticles synthesis for aquatic pathogen control

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**Abstract**. In this study, we used bacterial strain *Serratia nematodiphila* for the synthesis of silver nanoparticles using optimized biomass growth. In this RSM study the variables such as sodium sulphate (g / L) (0.5, 1, 1.5), magnesium sulphate (g /L) (0.3, 0.5, 0.7), pH (6.4, 7.4, 8.4.), temperature (25, 30, 35$^0$C) and Sodium lactate, Peptone have been used for the maximum production of biomass. We got very good a result for the silver nanoparticles was confirmed using UV-vis spectrophotometer and transmission electron microscope. Finally, we concluded that the using of RSM for nanoparticles synthesis may use in industrial biotechnology and related technologies for large scale production.

## 1. Introduction

Nanoparticles are having the wide variety of applications in biomedical field [1], [2]. In that silver nanoparticles are having very good properties such as shape, size and biocompatibility are the major reason for its biomedical applications [3]. The wide variety of biological resources has been used for the synthesis of silver nanoparticles such as fungus, bacteria, plants and marine algae etc. [4], [5], [6], [7]. The nanoparticles may involve in the biomedical applications like diagnosis and treatment of various health complications such as biosensor for using localized surface plasmon resonance (LPSR) for the identification of serum p53 in neck and head squamous cell carcinoma [8]. Basically a silver nanoparticle acts an antimicrobial agent like antibacterial on *Bacillus subtilis, Klebsiella planticola, E. coli, Staphylococcus aureus and Klebsiella pneumoniae*, [9] [10] antiviral agents, antifungal activity on *Aspergillus niger, Aspergillus fumigatus, Candida albicans, Aspergillus flavus and Fusarium sp* and it may use as a wound healing agent and controlling of dermatophytes [11], [12] and anticancer activity against lung, liver cancer cell line and multidrug-resistant cancer [13], [14]. The bacteria used for the silver nanoparticles synthesis is *Serratia nematodiphila* a sulfur reducing bacteria isolated from industrial wastewater. It is coming under the Order of Enterobacterialesand the family is Enterobacteriaceae.
RSM play a major role in enzymes production such as cyclodextrin lucanotransferase by *Bacillus stearothermophilus* HR1 [15], linolenic acid in Mortierella ramanniana var. ramanniana [16]. Nattokinase production by *Bacillus subtilis* [17], cyclodextrin glycosyltransferase production from

*Klebsiella pneumoniae*. [18], alkaline protease from *Bacillus horikoshii* [19], alkaline protease by *Bacillus sp*. [20] were produced previously.

In this present investigation, we used optimized the growth of industrially important microbe *Serratia nematodiphila* and it was used for the synthesis of AgNPs. The AgNPs characterized by UV - vis spectrophotometer and transmission electron microscope. The antibacterial activity of silver nanoparticles analyzed against *Bacillus cerus, Staphylococcus aureus, Streptococcus sp, E. coli and Salmonella sp*.

## 2. Materials and Methods

The chemical company effluent was collected and serially diluted from the isolation of bacteria. The isolated bacterium was biochemically identified using bergys manual.

### *2.1 Response Surface Methodology*

The total of Six parameters was included for selection, by each variable represented at three levels (-1, 0, +1). The variables were as follows: Sodium Sulphate (g/L) (0.5, 1, 1.5) (X1); Mg sulphate (g/L) (0.3, 0.5 0.7) (X2), pH (6.4, 7.4, 8.4.) (X3), Temperature (25, 30, 35 0C) (X4) and Sodium lactate (g/L) (X5), Peptone (g/L), (X6) at dissimilar concentrations of above nutrient are designed by design expert 7.0.1. For the selection of important variables for optimization of maximum biomass by bacterial strain *Serratia nematodiphila* variety of phsico-chemical factors such as Temperature (25º, 30º, 35º) and pH (6.4, 7.4, 8.4) at different concentrations of above nutrient agar designed.

### *2.2 Biosynthesis and characterization of AgNPs*

The bacterial biomass of *S. nematodiphila* added in the 1 mM of $AgNo_3$ and kept in shaker for and the colour change was observed. The UV-vis spectroscopic observation was taken from 300 nm to 600 nm periodically. After the completion of the reaction, the particle was purified using centrifugation and dried for nanoparticles powder preparation. The prepared powder is characterized for morphological analysis by TEM.

### *2.3 Antibacterial activity of AgNPs*

The antibacterial activity of biosynthesized AgNPs were analyzed against water pathogens like *Bacillus cerus, Staphylococcus aureus, Streptococcus sp, E. coli and Salmonella sp.* the antibacterial activity was conducted by agar well diffusion assay in Muller Hinton agar medium. The procedure was followed based on our earlier studies [21].

## 3. Results and Discussion

### *3.1 Isolation and Identification of bacterial strain*

In this study used the strain was isolated from chemical company effluent and saltpan soil. The isolates were morphologically and biochemically characterized as *Serratia nematodiphila* (chemical company effluent). *S. nematodiphila* produce red pigment. *Serratia nematodiphila* was gram positive, rod shaped and non-motile bacteria. Pure separate colonies were obtained and characterized as *Serratia nematodiphila* to identify from MTCC and maintain at the laboratory, Table 1.

**Table 1.** Cultural and Biochemical Characteristics Analysis

| **S. No** | **Biochemical Tests** | ***Serratia nematodiphila*** |
|---|---|---|
| 1 | Gram staining | Negative |
| 2 | Spore staining | Negative |
| 3 | Motility | Positive |
| 4 | Growth at 15 ºC | Positive |

| | | |
|---|---|---|
| 5 | Growth at 25 ºC | Positive |
| 6 | Growth at 37 ºC | Positive |
| 7 | Growth at 42 ºC | Positive |
| 8 | Growth at pH 5.2 | Positive |
| 9 | Growth at pH 8.0 | Positive |
| 10 | Growth at pH 9.0 | Positive |
| 11 | Growth on Nacl 12% | Positive |
| 13 | Growth on Nacl 15% | Positive |
| 14 | Growth on Nacl 17% | Positive |
| 15 | Growth on Nacl 10% | Negative |
| 16 | Starch Hydrolysis | Positive |
| 17 | Gelatin liquefaction | Positive |
| 18 | Casein Hydrolysis | Positive |
| 19 | H2S Production | Negative |
| 20 | Indole | Negative |
| 21 | Methyl Red | Negative |
| 22 | Voges Proskauer | Positive |
| 23 | Catalase | Positive |
| 24 | Oxidase | Negative |
| 25 | Urea | Negative |
| 26 | Nitrate Reduction | Positive |
| 27 | Arabinose | Positive |
| 28 | Galactose | Positive |
| 29 | Glucose | Positive |
| 30 | Mannitol | Positive |
| 31 | Raffinose | Negative |
| 32 | Salicin | Positive |
| 33 | Xylose | Positive |
| 34 | Sucrose | Positive |
| 35 | Rhamnose | Negative |

| | | |
|---|---|---|
| 36 | Meso-inosital | Positive |
| 37 | Fructose | Positive |

*3.2 Optimization studies*

The present investigation sulfur reducing media were carried out the highest growth in the bacterial biomass for *S. nematodiphila*. The six significant variables Sodium Sulphate (g/L), Mg sulfate (g/L), Temperature (°C), pH, Sodium lactate (g/L), Peptone (g/L), were further optimized using RSM.

$$Y = 1.485 + 0.051X_1 + 0.00.X_2 - 0.007X_3 + 0.033X_4 + 0.026X_5 + 0.016X_6 - 0.187X_1^2 - 0.038X_2{}^2 - 0.157X_3{}^3 - 0.2372X_4{}^2 - 0.2722X_5^2 + 0.1406X_6^2 - 0.004X_1X_2 + 0.0266X_1X_3 + 0.042X_1X_4 + 0.096X_1X_5 + 0.0017X_1X_6 + 0.000X_2X_3 + 0.009X_2X_4 - 0.001X_2X_5 + 0.0005X_2X_6 + 0.007X_3X_4 - 0.015X_3X_5 - 0.016X_3X_6 + 0.0615X_4X_5 + 0.0126X_4X_6 + 0.00X_5X_6 \quad (1)$$

The table 2 shows the calculated are listed co-efficient of regression model. The ANNOVA showed that the regression statistically significant at 99 % ($p<0.05$) confidence level analysed by the statistical significance of the model equation. The ANOVA for bacterial biomass production specified that the "F-value" of the model was 449.48, 138.2 by *S. nematodiphila* suggesting that the model was highly significant. The probability values (Prob > F) are less than 0.0001. The $R^2$-value of 0.9942, 0.99311given by regression is in reasonable agreement with the Adj - R squared of 0.9977, 0.9859 using the optimization of biomass activity from *S. nematodiphila,* "Adeq Precision" measures the signal to noise ratio.

**Table 2.** Regression study for the maximum biomass of *S. nematodiphila* for quadratic response surface model fitting (ANOVA)

| **Sources** | **Sum of Squares** | **df** | **Mean Square** | **F - Value** | **p-value Prob > F** |
|---|---|---|---|---|---|
| Model | 1.485 | 27 | 0.0781 | 860.3 | < 0.0001* |
| $X_1$-NaSO4 | -0.051 | 1 | 0.062 | 690 | < 0.0001* |
| $X_2$-MgSO4 | -0.0000 | 1 | 2.02 | 0.22 | 0.7320 |
| $X_3$-pH | 0.007 | 1 | 0.001 | 13 | 0.0127* |
| $X_4$-Temperature | -0.0331 | 1 | 0.0264 | 290 | < 0.0001* |
| $X_5$-Na Lactate | -0.026 | 1 | 0.0170 | 187 | < 0.0001* |
| $X_6$-Peptone | -0.016 | 1 | 0.0065 | 72.3 | < 0.0001* |
| $X_1X_2$ | -0.004 | 1 | 0.0001 | 1.6 | 0.3489 |
| $X_1X_3$ | 0.0266 | 1 | 0.0068 | 75.3 | < 0.0001* |
| $X_1X_4$ | -0.042 | 1 | 0.029 | 324.8 | < 0.0001* |
| $X_1X_5$ | -0.096 | 1 | 0.074 | 824.8 | < 0.0001* |
| $X_1X_6$ | 0.0017 | 1 | 2.45 | 0.26 | 0.7059 |
| $X_2X_3$ | -0.000 | 1 | 6.13 | 0.06 | 0.8502 |
| $X_2X_4$ | 0.009 | 1 | 0.0007 | 8.16 | 0.0457 |
| $X_2X_5$ | 0.0011 | 1 | 2.26 | 0.24 | 0.7172 |
| $X_2X_6$ | 0.0005 | 1 | 0.0000 | 0.02 | 0.9140 |
| $X_3X_4$ | -0.007 | 1 | 0.0004 | 4.7 | 0.1199* |
| $X_3X_5$ | -0.015 | 1 | 0.001 | 21.5 | 0.0021* |
| $X_3X_6$ | 0.016 | 1 | 0.004 | 49.8 | < 0.0001* |

| | | | | | |
|---|---|---|---|---|---|
| $X_4X_5$ | -0.0615 | 1 | 0.030 | 333.2 | < 0.0001* |
| $X_4X_6$ | 0.0126 | 1 | 0.001 | 14 | 0.0106* |
| $X_5X_6$ | -0.000 | 1 | 0.000 | 0.0 | 0.9569 |
| $X_1^2$ | -0.187 | 1 | 0.036 | 3997 | < 0.0001* |
| $X_2^2$ | 0.038 | 1 | 0. 0.014 | 164 | < 0.0001* |
| $X_3^2$ | -0.157 | 1 | 0.025 | 2803 | < 0.0001* |
| $X_4^2$ | -0.2372 | 1 | 0.578 | 6375 | < 0.0001* |
| $X_5^2$ | -0.2722 | 1 | 0.762 | 8397 | < 0.0001* |
| $X_6^2$ | -0.1406 | 1 | 0.203 | 2242 | < 0.0001* |
| Residual | 0.0023 | 26 | 9.08 | | |
| Lack of Fit | 0.0023 | 21 | 0.0001 | | |
| Pure Error | 0.0000 | 5 | 0 | | |
| Cor Total | 2.111 | 53 | | | |

***Values of "Prob >F" less than 0.0500 indicate model terms**

The counter plots represent the maximum biomass activity (1.942 (OD-ABS) with NaSO4 (0.10 to 0.20 g/L) and Mg SO4 (0.10 to 0.20 g/L) can be clearly shown Fig. 1 a & b. Optimum level of degradation (1.942 (OD-ABS) was at NaSO4 (0.15 g/L) and Mg SO4 (0.15 g/L) Fig. 1 (a). the bacterial culture kept at internal osmotic pressure at about 0.15 g/L solution of NaSO4. The contour plot represents maximum biomass activity against peptone and NaSO4 shows the biomass activity 1.166 at a particular range of peptone (0.5 to 0.7 g/L) and NaSO4 (1.25 to 1.65 g/L) is clearly shown in Fig. 1 (f) & 6 (f). The optimum level of biomass activity occurs with 98% at Peptone (0.15 g/L) and NaSO4 (0.15 g/L) calculated by derivatization of the equation and by solving the inverse matrix. RSM which represents the maximum biomass activity 100% at pH (6 to 7) and temperature (25 $^0$C to 35$^0$C) is shown figure 1 (c). The Optimization level of Temperature (30$^0$C) and pH (6.5) were determined at maximum biomass activity. As shown in Fig. 1 (d) the maximum biomass activity temperatures (25 to 35$^0$C) and Na lactate (0.35 to 0.45 g/L). Optimization level of temperature (30$^0$C) and Na lactate (0.4 g/L) were determined for maximum biomass activity. Temperature exerts an important regulatory influence on the rate of metabolism [17]. A optimum level of Na lactate (0.4 g/L) and peptone (0.15 g/L ) showed the maximum biomass activity as 1.485. The concentration of Na lactate in minimal medium Fig. 1 (e) was varied from (0.35 to 0.45 g/L) and there is no considerable increase in the biomass activity beyond 0.4 g/L. Increasing the concentration of Na lactate from 0.35% to 0.45% g/L increased the biomass activity from 0.892 to 1.485. 3 D plot representing maximum bacterial mass activity (0.860 against MgSO4 (0.10 to 0.20 g/L) and pH (6 to 7) were determined. Optimization level of Mg SO4 (0.15) and pH (6.5) were determined at maximum biomass activity, Fig. 1(b) & 6 (b) as (1.952 (OD-ABS), (1.485(OD-ABS) for *S. nematodiphila*.

*3.2.1 Perturbation plot*

Fig. 2 shows that each nutrient used in the present study has it individual effect on maximum biomass activity of *S. nematodiphila*. Herein, the sodium lactate and peptone play a significant role in the growth of *S. nematodiphila*, when compare to other variables. The perturbation plot of *S. nematodiphila* biomass also exhibits except sodium lactate the other variables such as magnesium sulphate, sodium sulphate have no significant effect on the biomass growth. The maximum *S. nematodiphila* biomass growth yield was 1.485 (Optical density-OD) and the optimized media composition was (g/L) sodium sulphate – 0.15, magnesium sulphate 0.15, pH – 6.5, temperature – 30, sodium lactate – 0.4 and peptone – 0.15. The major objective of the RSM is to optimize the growth of the bacteria *S. nematodiphila* for enhance synthesis of metal nanoparticles. Based on the results obtained from Box-Benhken design experiments the sodium lactate and peptone is the main nutrient in the media used for improved growth of biomass and it leads to the enhanced synthesis of silver nanoparticles by using *S. nematodiphila*.

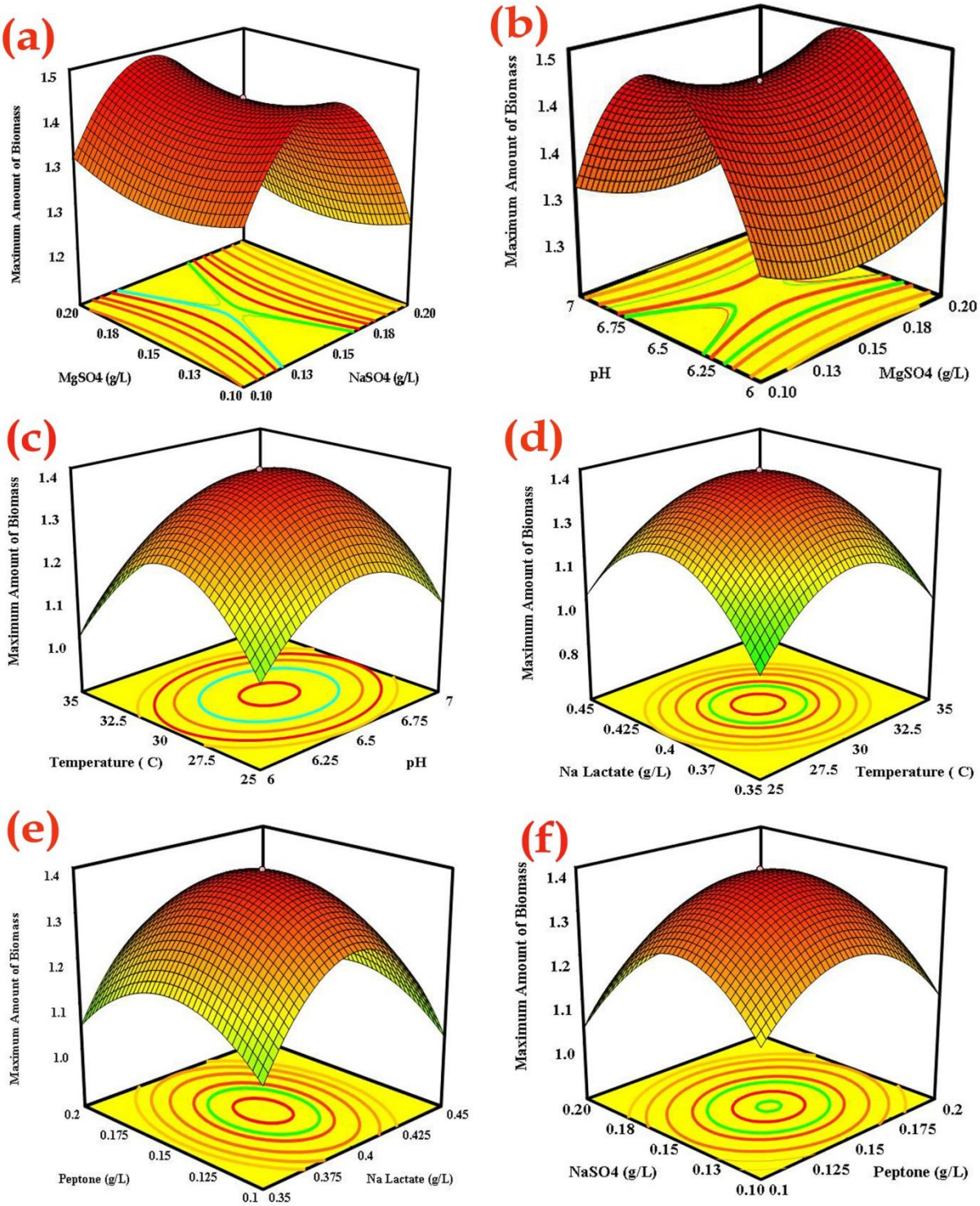


**Figure 1.** Maximum bacterial mass activity *(S. nematodiphila)* on 3- D graphics for response surface Optimization versus (a) $NaSO_4$ and $MgSo_4$ (b) pH and $MgSO_4$ (c) Temperature and pH (d) Sodium lactate and Temperature (e) Peptone and Na lactate (f) $NaSO_4$ and peptone

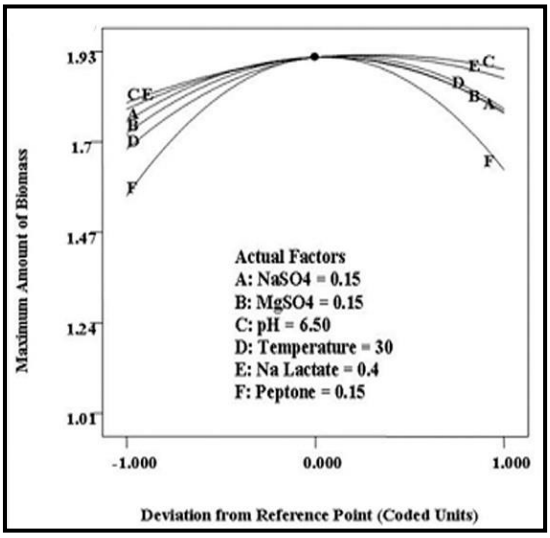


**Figure 2.** Perturbation graph of 5(g) *S. nematodiphila*

*3.3 Biosynthesis of AgNPs using optimized Serratia nematodiphila*

After the addition of silver nitrate with bacterial biomass, the color was changed into yellowish to brownish shows synthesis of silver nanoparticles. When compared to the normal broth, optimized biomass broth shows the very good peak in UV-vis spectroscopic absorbance shown in figure 3. The UV-vis spec shows surface plasmon resonance at 420 nm confirms the silver nanoparticles synthesis [2].

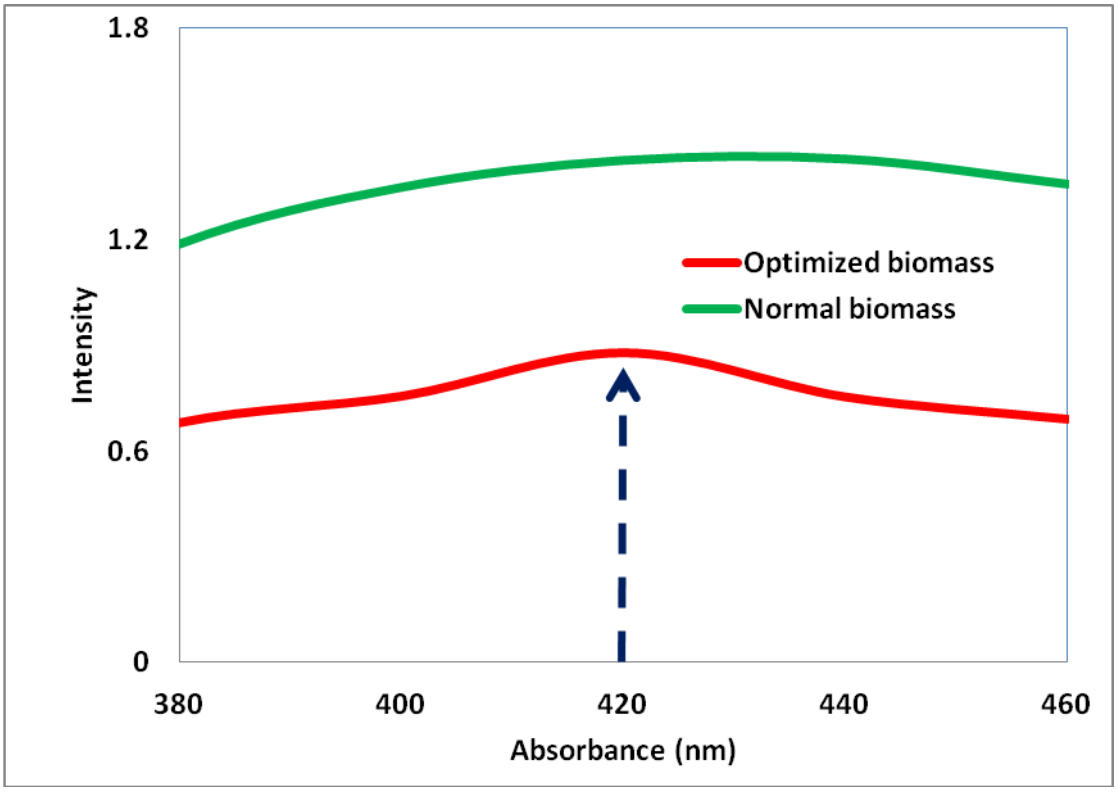


**Figure 3.** UV-vis spectroscopic analysis of silver nanoparticles synthesized using *S. nematodiphila*

*3.3.1 Transmission electron microscope*

TEM is the very good technique used for the analysis of nanoparticles size and shape [22]. The different shapes of silver nanoparticles observed In the TEM image shown in figure 4. The shapes like

spherical, triangle, pseudo spherical and rectangle were observed in the image. The size of the silver nanoparticles is from 20 nm to 40 nm. In the background silver nanoparticles, the images some of the ash color particles were found may be the metabolites present in the bacterial biomass [23].

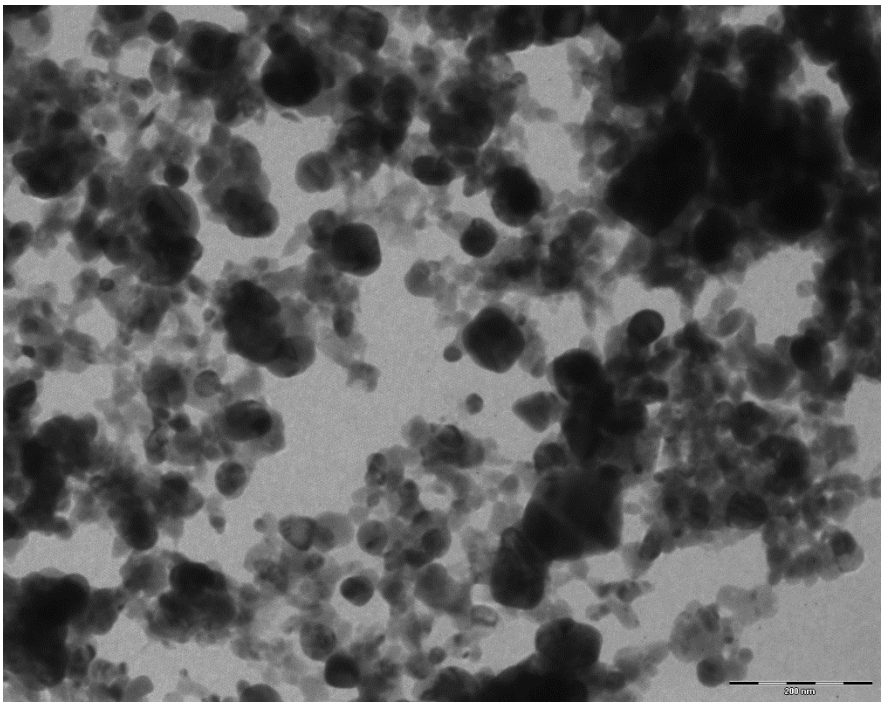

**Figure 4.** TEM image of silver nanoparticles synthesized using optimized biomass growth

*3.4 Antibacterial activity of AgNPs against Aquatic pathogens*

The synthesized silver nanoparticles were used for the controlling of growth of pathogenic bacteria isolated from the aqueous sample. The silver nanoparticles control the growth of pathogenic microbes in the Muller Hinton agar containing the medium. The zone of inhibition was represented in the clustered column graph (Fig 5). Mostly the increased concentration shows the highest zone of inhibition but in the Bacillus cerus 50 µl shows more inhibition than 100 µl of silver nanoparticles solution. The culture isolated from the aqueous are mostly the disease-causing pathogens like typhoid, fever, stomach problems etc. the silver nanoparticles action on the disease-causing bacterial culture is glycan strands decomposition, accumulation in bacterial membrane, deactivation of bacterial enzymes and inhibit deoxy ribonucleic acid synthesis [23,24,25].

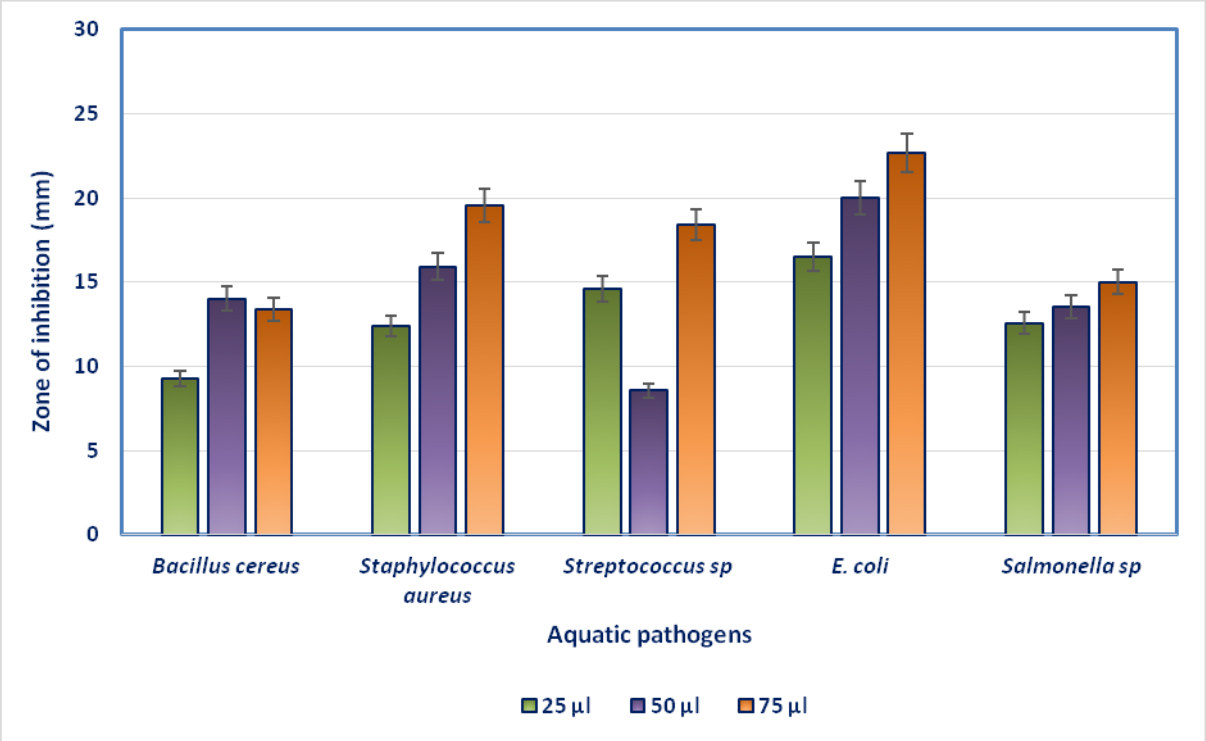


**Figure 5.** Antibacterial activity of silver nanoparticles

## 4. Conclusion

Synthesis of silver nanoparticles using optimized biomass of industrially important microbe *Serratia nematodiphila* was performed. For the optimization process response surface methodology was applied to the production of maximum bacterial biomass production. Herein maximum silver nanoparticles were produced when compared with normal biomass proved by UV-vis spectrophotometer. The antibacterial activity of silver nanoparticles against aquatic pathogenic bacteria shows the very good zone of inhibition. Based on the results, this method may use for high production and commercialization of silver nanoparticles in industrial level for various applications in biomedical.

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