Current Trends in Technology and Sciences
Volume : 1, Issue : 1 (July-2012)

Kinetics of Biosynthesis of Silver Nanoparticles Using Fusarium Oxysporum

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Abstract - The emergence of nanobiotechnology has exposed the greener methods of preparation and application. Many theoretical and experimental works have been conducted in the biosynthesis of various nanomaterials and nanocomposites. Biologically synthesised nanomaterials are exhibiting excellent, unique and optimised properties. Micro organisms play an important role in the detoxification of metals through the reduction of metal ions. This process can be exploited for biosynthesis of NPs. Experiments were conducted by varying the concentrations of silver ions for analysing the formation of silver nanoparticles by *Fusarium Oxysporum*. The analysis has been done for extracellular and intracellular in the growing cell and whole cell sysytems. Silver nanoparticles formed by *Fusarium Oxysporum* was found to be purely extracellular. In this paper, from the experimental analysis, an attempt is being made to suggest a mechanism and elucidate the kinetics for the formation of silver nanoparticles.

Keywords - Biosynthesis, Fusarium Oxysporum, Kinetics, Silver nanoparticles

1. INTRODUCTION

over, biosynthesis has recently attracted attention mainly because of its clean, reliable, non-toxic and ecofriendly nature [14]. Since the reaction rate is low, biosynthesis offers better control over the growth of nanoparticle crystals and their stabilization [15]. As the surface area of materials increases during their evolution from macro to nanoscale, they exhibit high surface reactivity and biocompatibility which promise their potential *in vivo* medicinal applications [7]. Medicinal applications of nanotechnology are in the areas of molecular imaging, cancer detection and therapy inside the body, *in vivo* sensors, X-ray absorbers. Another key area where nanotechnology promised its astonishing application is in agriculture and food. Nanotechnology tools are used during cultivation, production, processing and package of food. Thus the foot print of nanotechnological applications can be seen in every walk of life. Nanosilver is the most cited nanomaterial ever used and also it is emerging as one of the fastest growing nanomaterials with variety of applications. It has been known for some

The word "Nanotechnology" was coined by Taniguchi in 1974 to describe precision manufacturing materials at the nanometer level [27] which refers to the synthesis, manipulation and control of matter at nano dimensions that will make most products lighter, stronger, cleaner, less expensive and more precise. It is the evolutionary understanding of materials [20]. Eventhough nanotechnology is at its infancy, its rapid growth is opening up a floodgate of opportunities. There are unusual changes in all properties of materials when it is being converted into its nanosize, which will improve their stability and functionality [24], [12], [6]. Some of the physical properties exhibited by nanomaterials are due to large surface atom, large surface energy, spatial confinement and reduced imperfections [22]. Currently there is an evergrowing demand for nanoparticle synthesis through processes which are less malevolent to the environment. The synthesis of nanomaterials by nature has contributed very much to the development of research based biosynthesis of nanoparticles. Biosynthesis requires only mild experimental conditions and also it can give higher productivity at a lower cost, if its full potential is being exploited, than chemical or physical methods. Biological methods offer higher stability of nanoparticles in liquid suspension [18]. More

time that silver is highly toxic to a wide range of bacteria and thus silver based compounds have been used extensively in bactericidal applications [21]. This property of silver has caused great interest as a new resistant to bacteria which are harmful to public health. Reductases producing by fungi are responsible for the conversion of silver ions to SNPs [1]. Silver in the form of nanoparticles is even more effective because of their high surface to volume ratio so that a large proportion of silver atoms are in direct contact with their environment. Nanoparticles are sufficiently small enough to pass through outer cell membranes and enter cells' inner membranes. A recent medical study showed that only Silver particles with sizes less than 10 nm were able to enter cells and disrupt those [7].

Biosynthesis of nanoparticles can be divided mainly into two categories depending on the location of formation of nanoparticles as either extracellular formation (nanoparticles are forming outside the cell) or

intracellular formation (nanoparticles are forming inside the cell) [13]. Eventhough intracellular process offers good control over the size and shape of the nanoparticles, extracellular formation of nanoparticles is more advantageous of the fact that it makes the downstream processing much easier [4]. The applications of the nanoparticles will be better understood if produced extracellularly [1].

2. Materials and Methods

Fusarium Oxysporum MTCC ACC NO. 284 was grown in the growth medium proposed by Holker et al. [9]. The experiments were conducted for the following cases. In the case of Growing Cell Systems (GCS), silver nitrate (Merck, India, 99.5 % pure) was added into the growth medium at the time of inoculation and the formation of silver nanoparticles was observed. In the latter case, i.e. Whole Cell Systems (WCS), the addition of silver nitrate was made after allowing the organism to grow fully and the growth was observed and analysed.

In both the cases, the extracellular and intracellular formations of silver nanoparticles were investigated in detail at room temperature and at 150 rpm. Separate experiments were carried out in four different concentrations of silver ions, viz., 10 mM, 5 mM, 1 mM and 0.1 mM for whole cell systems and 10 mM, 1 mM, 0.5 mM and 0.1 mM for growing cell systems. The organism present in the growth medium was capable of reducing Ag+ ions to Ago ions in five days and leads to the formation of silver nanoparticles [1]. In WCS 0.1 mM and in GCS 10 mM were not showing any formation of SNPs. All samples were analyzed in a UV visible Spectrophotometer with growth medium as the blank, while intracellular samples were analyzed with buffer solution as blank. Control was also analyzed without the addition of silver nitrate. TEM was carried out using a JOEL 3011, 300 kV instrument with an ultra-high resolution pole piece.

The quantitative estimation of concentration silver in the medium is done by using spectrophotometric method (Vogel).

3. Results

In WCS, formation of silver nanoparticles is easily perceptible due to the change of colour of the solution while the colour of the control run remains unchanged during the course of the reaction [17]. Colour change of the solution from pale yellow to brownish red is the primary observation of the formation of NPs. All spectra [17] are exhibiting peak at 450 nm (figures not shown) (due to Surface Plasmon Resonance which is the excitation of surface plasmons [1] when being hit by light of particular wavelength [24] without any shift in the maximum wavelength. This indicates longitudinal Plasmon vibrations [6]. It appears to suggest strongly with all experimental analysis that the synthesis of silver nanoparticle formation by *Fusarium Oxysporum* is purely extracellular in the case of WCS. In GCS, there is formation of SNPs but it is not purely extracellular and also not stable as in the case of WCS [16] [17].

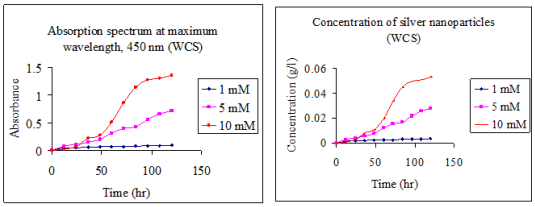

Fig.1. Absorbance and Concentration of SNPs at maximum wavelength (450 nm) in WCS.

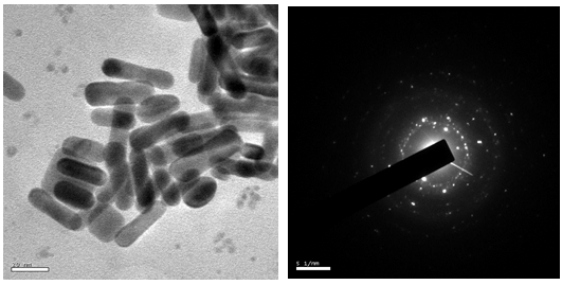

Fig.2. HRTEM image of silver nanoparticles (WCS), SAED pattern recorded on the sample.

Thus from the Fig 1, the absorbance or the concentration of silver nanoparticles is increasing with the increasing initial substrate concentration. Thus the concentration of silver nanoparticles is dependent on the initial concentration of substrate in the positive direction. Separate run has been conducted for 15 mM silver ions in the case of WCS where there was no indication of the formation of SNPs in the primary observation (there was no colour change for the solution). This may be due inability of the conversion of silver ions to SNPs by the enzymes when the concentration of silver ions is increased under the prevailing operating conditions.

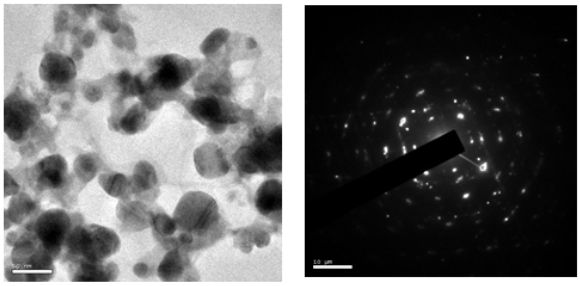

Fig.3. HRTEM image of silver nanoparticles (GCS), SAED pattern recorded on the sample.

3.1 Effect of Initial Silver Ion Concentration in The Nanoparticle Formation

The initial concentration ratio of silver ions to the cell free extract in the reaction mixture is the key factor in controlling the size of these nanoparticles. From Fig 1, in the case of 10 mM silver ions, the initial rate of formation of silver nanoparticles is low so that enough opportunity is there to manipulate the reaction conditions to get products with desired properties. Thus according to the present experimental results, 10 mM is the optimum initial silver ion concentration for the production of silver nanoparticles with fair monodispersion.

Fig. 2 illustrates a typical Transmission Electron Micrographs (TEM) of the samples under high resolution. In WCS the particles exhibit rod like structure with a length of 25 nm and width of 5 nm. The particles are fairly monodispersed in nature. The SAED (Selected Area Electron Diffraction) pattern, shows concentric rings with intermittent bright dots, indicates that the particles are highly crystalline in nature.

In GCS (Fig 3), particles are polydispersed in nature with a size range from 10 nm to 70 nm. Moreover, the particles are showing some sort of instability since they have a tendency to form agglomerates.

4. DISCUSSIONS

Researchers are trying to achieve such a technology for nanoparticle synthesis which minimizes the synthesis time, reduce the size and with processes which are non hazardous. The aim is to manipulate the rate of formation of nanoparticles, their size, shape, monodispersity and composition. These can be achieved by altering key growth parameters of the nanoparticles like pH, temperature, substrate concentration and exposure time [14]. Reduction of metal ions to nanoparticles is occurred by enzymatic process [1].

To achieve a better control over size and polydispersity of nanoparticles we should deal with the kinetics of synthesis of nanoparticles. From the experimental analysis, a possible mechanism is proposed and hence the kinetics for the synthesis of silver nanoparticles by *Fusarium Oxysporum* is discussed in the following session.

4.1 Whole Cell Systems

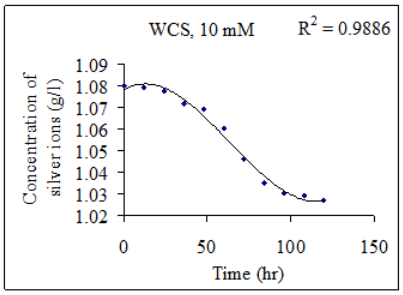

Fig.4. Concentration of silver ions (10 mM, WCS.)

When the concentration of the substrate is plotted against. time it is observed that the concentration of silver ions is decreasing with time (Fig. 4). This advocates the reduction of silver ions to SNPs.

4.1.1 Mechanism

In the case of 10 mm silver ions (Fig. 1), during the first two days the intensity of the silver nanoparticles increases almost linearly with time and then grows exponentially until the graph flattens out after five days. This indicates that the reaction follows a Michaelis Menten type of mechanism wherein it initially exhibits a pseudo zero order kinetics and then follows higher order kinetics with respect to the concentration of the reactants. In the beginning, the concentration of silver ions was significantly higher; therefore its consumption during the course of its reduction was practically negligible, rendering a linear dependence of the concentration of product with time. As the reaction proceeds, the concentration of silver ions comes down drastically. Now its concentration in the rate equation is no longer negligible compared to other reactants and that is indeed manifested in the nonlinearity of the curve.

Enzyme-catalyzed reactions usually follow Michaelis-Menten kinetics where the rate of the reaction has a sigmoidal dependence on substrate concentration. This is a type of activation effect since as the substrate concentration increases the reaction rate also increases. Here, a deviation from the usual behaviour is seen [2]. Sometimes when substrate concentration is high the rate of the enzyme catalysed reaction will be diminished. This is called substrate inhibition. A plot of rate of substrate degradation, v = dS/dt against. substrate concentration, S is showing a uniform trend in all initial substrate concentrations as in figures below. When we observe the above case (Fig 5), eventhough the R^2 value is high in the case of 10 mM silver ions, (R^2 is 0.9281 for 1 mM and 0.8768 for 5mM, figures not shown), we can see clearly that rate is decreasing at high substrate concentrations in all cases.

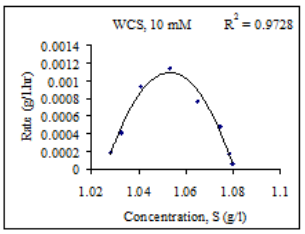

Fig.5. A plot of rate vs. concentration in WCS

The autocatalytic feature of this type of kinetics can also be modelled using Michaelis Menten approach. However, from the R^2 values, it is clear that the suggested mechanism is fitting more to the data with an initial silver ion concentration of 10 mM.

Reaction steps at equilibrium:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \qquad ((1)$$

$$ES + S \underset{k_4}{\overset{k_3}{\rightleftarrows}} ES_2 \qquad (2)$$

Slow step:

$$ES \xrightarrow{k_5} E + P \qquad (3)$$

where $$\frac{k_1}{k_2} = K_1, \frac{k_3}{k_4} = K_2 \qquad (4)$$

Thus we get rate,

$$v = \frac{k_5 e_0}{1 + \frac{K_1}{S} + \frac{S}{K_2}} \qquad (5)$$

where e_0 is the total enzyme concentration and v is the rate of degradation of substrate.

In the case of Michaelis- Menten approach, the mechanism is explained in two steps. As a starting point, it is assumed that the enzyme E and the substrate S

combine to form a complex ES as in equation 1, which then dissociates into product P and free enzyme E as in equation 3. However, here, a second substrate molecule can bind to the enzyme. When S joins the ES complex, an unreactive intermediate, ES2 results.

4.1.2 Deduction of Parameters

If the enzyme concentration is known, e_0, k_5 can be determined by varying e_0.

Also we have

$$\frac{1}{v} = \frac{1 + \frac{K_1}{S} + \frac{S}{K_2}}{k_5 e_0} \quad (6)$$

At large substrate concentration,

$$\frac{1}{v} = \frac{S}{K_2 k_5 e_0} \quad (7)$$

Therefore, a plot of 1/v vs. S gives slope as $\frac{1}{K_2 k_5 e_0}$ and finally K_2 can be obtained from $C_{\max} = \sqrt{K_1 K_2}$..

4.2 Growing Cell Systems

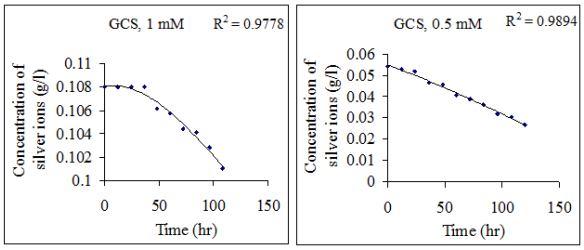

Fig.6. Concentration of silver ions in GCS

In growing cell systems the formation of nanoparticles is not showing any common trend. At a concentration of 1 mM silver ions, it is showing intracellular formation of silver nanoparticles, while in the case of other concentrations nanoparticles are forming outside the cells. (Fig 6)

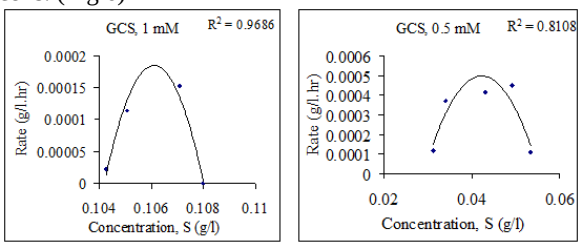

Fig.7. A plot of rate vs. concentration in, GCS.

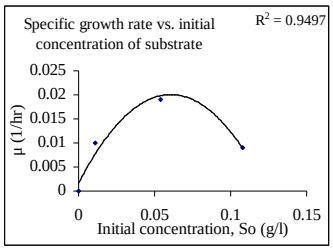

Fig.8. A plot of specific growth rate vs. initial substrate concentration in the case of growing cell systems

Also, in the cases of 1 mM and 0.5 mM silver ions, the intensity of the peak of the absorption spectrum is steadily increasing with time, but, in the case of 0.1 mM the peak intensity is increasing up to 48 hrs and then it is showing undulations. At 10 mm silver ions it is not showing any formation of nanoparticles [17] (Figures not shown). So it is difficult to propose a mechanism in this case despite from the Fig 7, the enzyme kinetics is likely to follow Michaelis Menten approach with substrate inhibition.

4.2.1 Microbial Growth Kinetics In GCS

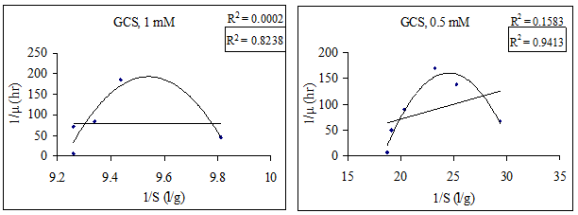

Fig.9. A plot of 1/μ vs. concentration of silver ions (GCS)

The relation between specific growth rate, μ, of a population of microorganisms and the substrate concentration, S is a valuable tool in biotechnology. This relationship is represented by a set of empirically derived rate laws referred to as theoretical models. These models are nothing but mathematical expressions generated to describe the behaviour of the system. The idea of microbial growth kinetics has been dominated by an empirical model originally proposed by Monod as follows.

$$\mu = \mu_{\max} \frac{S}{K_S + S} \quad (8)$$

where μ is the specific growth rate and is defined as

$$\mu = \frac{1}{X} \frac{dX}{dt} \quad (9)$$

where X is the biomass concentration.

$\mu_{\max}$ is the maximum specific growth rate and K_S is Monod's constant.

When a substrate inhibits its own biodegradation, the original Monod model becomes unsatisfactory. Among the substrate inhibition models, the Haldane's Equation (10) is most widely used.

According to this,

$$\mu = \frac{\mu_{\max}}{1 + \frac{K_s}{S} + \frac{S}{K_I}} \quad (10)$$

K_I is the inhibition constant.

According to [29], when μ is plotted against initial substrate concentration, Haldane's plot gives a parabolic profile. A similar trend is observed in our case also (Fig 8). Hence the microbial growth is following Haldane Kinetic Model [29]. So from a plot of 1/ μ vs. S, the parameters of Haldane's model can be deduced

4.2.2 Data Comparison With MONOD'S Model And Haldane's Model

The experimental data were tried with both Monod kinetic model and Haldane kinetic model by plotting 1/μ vs. 1/S (Fig 9). The curve and the mean square value in the box represent Haldane's model, while the straight line represents Monod's model. The value of the correlation coefficient (R^2 = 0.0002 for 1 mM and R^2 = 0.1583 for 0.5 mM) showed that the present data do not confirm well to Monod's model. The Monod's model only describes the dependence of biodegradation rate on the biomass concentrat. When the biodegradation exhibits inhibition, the Monod's model fails. The plot of 1/μ vs. 1/S is fitting best with curves as in Figures 15 and 16. The value of correlation coefficient (R^2 = 0.8238 for 1 mM and R^2 = 0.9413 for 0.5 mM) shows that the present data confirm well to the Haldane kinetic model as compared to Monod kinetic model.

5. Conclusions and Future Perspectives

It is already proved from the experiments that silver nanoparticles synthesis by Whole Cell Systems is better than by Growing Cell Systems [17]. The kinetics is more or less following Michaelis Menten Mechanism. However, more experiments have to be done to give a clear cut explanation on the mechanism of the SNP synthesis. If mechanism is known better control over size and polydispersity of NPs can be achieved. Another paramount important area where we have to give more emphasis is the elucidation of stability of the synthesized NPs. Detailed analysis is to be done for the separation and isolation of NPs and the proteins and the enzymes secreted by the micro organisms. Little is known about the environmental impact of the NPs to ecological system.

NPs have very important potential applications all walks of life. They have certain properties which makes them highly efficient in various applications and enhancing their demand in the market. These properties depend on the size, shape, composition, monodispersity and stabilty of the NPs. Now researchers are trying to cross the herculian task of enhancing the stability of the synthesized NPs with time. Synthesis of monodispersed nanoparticles is also equally important and demanding. Another most important area where we have not achieved full success is in the purification or isolation of NPs. For the acceptance of any technology for the nanoparticle synthesis, it should take minimum time for synthesis, nanoparticles should be stable and monodispersed and the process should be nonhazardous. Hence the old problem, silver, is now replaced with new challenges by the evolution of SNPs. What we are observing is only a tip of the iceberg, more explorations are yet to be done in this area.

6. References

[1] A. Ahmad, P. Mukherjee., S. Senapati, D. Mandal, M. I. Khan, R. and M. Sastry,."Extracellular biosynthesis of silver nanoparticles using thefungus *Fusarium Oxysporum"*, Colloids and Surfaces B: Biointerfaces, vol. 28, pp. 313-318, 2003.

[2] J. E. Bailey and D. F. Ollis.,Ed, "Biochemical Engineering Fundamentals", Second Edition, McGraw-Hill Inc., New York, pp. 115-117, 1986.

[3] D. S. Balaji, S. Basavaraja, M. D. Bedre, B.K. Prabhakar, and A. Ventaraman, "Extracellular biosynthesis of functionalized silver nanoparticles by strains of Cladosporium cladosporioides fungus", Colloids and Surfaces B: Biointerfaces, vol. 68, pp. 88-92, 2009.

[4] K.C. Bhainsa, and S.F. D' Souza, "Extracellular biosynthesis of silver nanoparticles using the fungus Aspergillus fumigates", Colloids and Surfaces B: Biointerfaces, vol. 47, pp. 160-164, 2006.

[5] G. Cao,Ed.,. "Nanostructures and Nanomaterials: Synthesis, Properties and Applications". Imperial College Press, London,2004.

[6] M.C. Daniel and D. Astruc,. "Gold Nanoparticles: Assembly, Supramolecular Chemistry, Quantum-size related properties and applications towards biology catalysis and Nanotechnology", Chemical Society Reviews, vol. 104, pp. 293-346, 2004.

[7] J. L. Elechiguerra, J.L. Burt, J.R.Morones, A.C. Bragado, X. Gao, H.H. Lara, and M.J. Yacaman, "Interaction of silver nanoparticles with HIV-1". Journal of Nanobiotechnology, vol. 3, pp. 1477-3155, 2005.

[8] M.A. Hayat.,"Colloidal Gold: Principles, Methods and Applications". Academic Press, California, 1989.

[9] U. Holker, R. M. Fakoussa, M. Hofer, "Growth substrates control ability of Fusarium Oxysporum to solubilise low-rank coal", Applied Microbiology and Biotechnology, vol. 44, pp. 351-355, 1995.

[10] J.Klaus, R. Jeorger, E. Olsson and C. Granqvist, "Bacteria as workers in the living factory: metal accumulating bacteria and their potential for material science", Trends in Biotechnology, vol. 19, pp. 15-20, 2001.

[11] M. F. Lengke, M. E. Fleet and G. Southam. "Biosynthesis of silver (I) nitrate complex"., Langmuir, vol. 23, pp. 2694-2699, 2007.

[12] L. N. Lewis. "Chemical catalysis by colloids and clusters"., Chemical Reviews, vol. 93,1993, pp. 2693.

[13] S. Mann, Ed.,. Biomimetic Materials Chemistry, VCH Newyork, 1996.

[14] P. Mohanpuria, N. K. Rana, and S. K. Yadav, "Biosynthesis of nanoparticles: technological concepts and future applications.", Journal of Nanoparticle Research, vol. 10, pp. 507-517, 2008.

[15] P. Mukherjee, M. Roy, B. P. Mandal, G. K. Dey, P. K. Mukherjee, J. Ghatak, A. K. Tyagi, and S. P. Kale., "Green synthesis of highly stabilized nanocrystalline silver particles by a non-pathogenic and agriculturally important fungus T. asperellum.", Nanotechnology, vol. 19, pp. 075103-075110, 2008.

[16] P. N. Praseetha, "Silver nanoparticle synthesis using Fusarium oxysporum" Twenty fifth National

Convention of Chemical Engineers, pp. 151-157, 2008.
[17] P. N. Praseetha and T. Panda, "Silver nanoparticle biosynthesis using Fusarium oxysporum: A comparative study in whole cell systems with growing cell systems", Global Journal of Engineering and Applied Sciences, vol. 1, pp. 113-116, 2011.
[18] R. Y. Parikh, S. Singh, B. L.V. Prasad, M. S. Pathole, M. Sastry and Y. S. Shouche, "Extracellular synthesis of crystalline silver nanoparticles and molecular evidence of silver resistance from Morganella sp.: Towards understanding biochemical synthesis mechanism.", Chemical and Biochemical Engineering Quarterly, vol. 9, pp. 1415-1422, 2008 .
[19] C. P. Poole and F. J. Owens, Ed., "Introduction to Nanotechnology", Wiley, pp. 1-1, 2008.
[20] T. Pradeep, Ed., "Nano: The Essentials – Understanding Nanoscience and Nanotechnology", Tata McGraw-Hill, pp. 3-15, 2007.
[21] M. Rai, A. Yadav and A. Gade. "Silver nanoparticles as a new generation of antimicrobials". Biotechnology Advances, vol. 27, pp. 76-83, 2009.
[22] N. Saifuddin, C. W. Wong and A. A. N. Yasumira. "Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation". E- Journal of Chemistry, vol. 6, pp. 61-70, 2009.
[23] O. V. Salata, "Applications of nanoparticles in biology and medicine"., Journal of Nanobiotechnology, vol. 2, pp. 3, 2004.
[24] G. Schmid. "Large clusters and colloids: Metals in the embryonic state". Chemical Reviews, vol. 92, pp. 1709-1727, 1992.
[25] S. Schultz, D. R. Smith, J. J. Mock and D. A. Schultz, "Single-target molecule detection with non bleaching multicolor optical immunolabels.", Proceedings of the National academy of Sciences of the United States of America, vol. 97, pp. 996-1001, 2000.
[26] I. Sondi and B. Salopek-Sondi, "Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for gram-negative bacteria"., Journal of Colloid and Interface Science, vol. 275, pp. 177-182, 2004.
[27] N. Taniguchi, "On the basic concept of Nanotechnology", Proc Intl Conf Prod Eng Tokyo, Part II. Japan Society of precision Engineering, 1974.
[28] V. I. Vogel (Ed.), "Textbook of Quantitative Inorganic Analysis", Longman Group Ltd, London,, pp. 162-163, 1978.
[29] Saravanan, P., Pakshirajan, K. and Saha, P, "Growth kinetics of an indigenous mixed microbial consortium during phenol degradation in batch reactor"., Bioresource Technology, vol. 99, pp. 205-209, 2008.