Preparation and characterisation of nanofibres from bio cellulose and neem-AgNP bio composites for wound healing

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Abstract: Bacterial cellulose (BC) is secreted by a few strains of bacteria and consists of a cellulose nanofibre network with unique characteristics. In this study, *Gluconoacetobacter xylinus* was used to isolate bacterial cellulose. Moreover, microbial cellulose has proven to be a remarkably versatile biomaterial and can be used in paper industry, electronics, wound healing and biomedical devices. The cellulose isolated from *G. xylinus* was confirmed by the biochemical tests. The parameters for the production of cellulose such as pH, temperature, carbon source, nitrogen source, and growth factor both static and shaking conditions were optimised. The cellulose obtained was observed by phase contrast microscope. The isolated bacterial cellulose is impregnated with silver nanoparticles synthesised by neem leaves extract. The bio cellulose and neem AgNPs were converted into nanofibres by electrospinning technique. The cellulose-PVA composite was analysed by SEM, zeta potential and Fourier transforms infrared spectroscopy (FTIR). This approach can be easily used in the large scale production of bio cellulose fibres loaded with silver nanoparticles. These fibres can be used in the wound dressing including use as a biomaterial for scaffolds in tissue engineering.

Keywords: bio cellulose; nanoparticles; neem leaves; PVA; scanning electron microscopy; SEM; zeta potential; Fourier transform infrared spectroscopy; FTIR.

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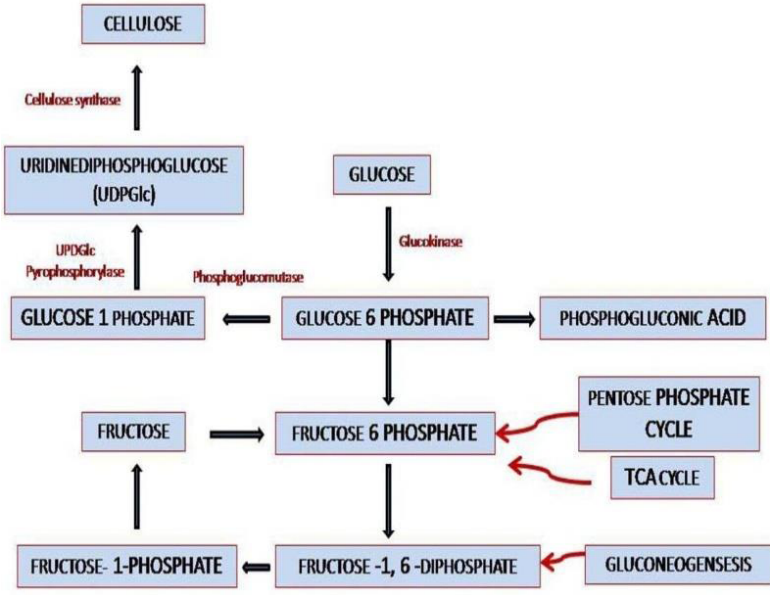
1 Introduction

Cellulose is a major biopolymer that finds enormous economic importance world-wide, it is the major constituent of cotton (over 94%) and wood (over 50%). These are used in many industries like paper, textiles, construction material, and cardboard for manufacturing purposes. Also, synthetic derivatives of cellulose like cellophane, rayon, and cellulose acetate are being used. Cellulose from bark of plants and cotton is derived from glucose, which is produced in the living plant cell, as a result of photosynthesis (Sherif and Keshk, 2014). Bacterial cellulose or bio-cellulose (BC) is produced by many species of microorganisms as an extracellular byproduct. BC is a superior biomaterial, possessing better qualities than other cellulose. BC has unique characteristics that differ from plant cellulose, which are water-holding capacity (over 100 times its natural state), degree of crystallinity, tensile strength, purity in a greater proportion, better elasticity, non-drying state, excellent biocompatibility, and it is free from other secondary components such as hemicellulose and lignin. These physical and mechanical qualities of BC are more useful than other materials as an alternative in food, biomedical and other industries (Amarnath and Henzert, 2013). Fibres of bacterial cellulose when compared to plant cellulose are about 100 times thinner thereby giving an advantage of being highly porous material giving potential to transfer of antibiotics and medicines into the wound which also serves as an efficient environmental barrier against external parameters. The molecular formula of bacterial cellulose $(C_6H_{10}O_5)n$ is the same as that of plant cellulose, but their physical and chemical features are different (Brown, 1886). The BC network structure comprises cellulose nanofibrils with 3–8 nm in diameter. The specific properties of BC such as the unique physical, mechanical properties nano-metric structure, with higher purity have led to a great number of commercial products(Klemm et al., 2001).

Synthesis of cellulose is catabolic process. The production of UDPGlc starts with carbon compounds by entering the Krebs cycle, gluconeogenesis, or the pentose phosphate cycle depending on the availability of carbon source (Brown, 2004). The cellulose precursor is UDPGlc. The pathway of cellulose involves glucose phosphorylation into glucose-6-phosphate (glu-6-PO_4) which is catalysed by glucokinase, followed by isomerisation into glucose-1-phosphate catalysed by phosphoglucomutase and then conversion into uridine diphosphoglucose (UDP-glc) by UDPGlc

pyrophosphorylase finally into cellulose catalysed by cellulose synthase (Jonas and Farah, 1998).

Figure 1 Mechanism of bacterial cellulose biosynthesis (see online version for colours)



Source: Brown (2004)

BC can be synthesised from the following species of bacteria, belonging to the genera *Gluconacetobacter* (formerly *Acetobacter*), *Agrobacterium*, *Aerobacter*, *Achromobacter, Azotobacter*, *Rhizobium, Sarcina, and Salmonella* and secreted outside of the cell (Shoda et al., 2005; Bilgi et al., 2016; Saeed et al., 2004). Production of cellulose from *Acetobacterxylinum* was first studied by Brown (1886). Gram-negative species like *Acetobacter*, *Agrobacterium*, *Achromobacter*, *Aerobacter, Sarcina, Azotobacter, Rhizobium, Pseudomonas, Salmonella* and *Alcaligenes* can produce cellulose. Cellulose is also synthesised by the Gram-positive bacterium *Sarcina ventriculi*, accounting for about 15% of the total dry cell mass. The most effective producers of cellulose are *A. xylinum, A. hansenii* and *A. pasteurianus* (Gromet-Elhanan and Hestrin, 1963; Park et al., 2003; Yoshino et al., 1996). *Acetobacter xylinum* produces two forms of cellulose such as ribbon like polymer, and the thermodynamically more stable amorphous polymer (Jones and Farah, 1998). The field of nanotechnology is one of the most active researches nowadays in modern material science and technology. Nanoparticles are fundamental building blocks of nanotechnology. The most important and distinct property of nanoparticles is exhibiting the larger surface area to volume ratio (Leela and Vevekanandan, 2008). Physical and chemical methods are more popular for nanoparticle synthesis, but the use of toxic compounds limits their application (Handy et al., 2008). The biosynthesis methods employing plant extracts have drawn attention as a simple and viable alternative to chemical procedures and physical methods, and bio reduction of silver ions to yield metal nanoparticles using living plants, geranium leaf, and neem leaf (Nagajyoti et al., 2011).

Azadirachta indica, commonly known as neem, belongs to *Meliaceae* family, and is well known in India and its neighboring countries for more than 200 years. It is one of the most versatile medicinal plants having a wide spectrum of biological activity and to name a few such as antifungal, antidiabetic, antibacterial, antiviral etc. Every part of the tree has been used as a traditional medicine for household remedy against various human ailments from antiquity (Hong et al., 2013). Synthesis of nanoparticles especially gold and silver can be done by using leaves extract of *Azadirachtaindica*. The advantage of using the neem leaves is that it is available abundantly and the antimicrobial activity from the obtained neem silver nanoparticles is enhanced by the reducing agent (Tran et al., 2013). Recently, the incorporation of metal nanoparticles on polymer nanofibres has attracted the interest of scientists and industries because the metal nanoparticles can endow the polymer nanofibres with distinctive properties, such as optical, electronic, catalytic, and antimicrobial properties (Mahouche-Chergui et al., 2013; Heness, 2012; Barakat et al., 2010).

Electrospinning is simple method used in fabricating synthetic and natural nanofibres and a low-cost method for making ultrathin diameter fibres. The electrospinning process is that a polymer solution is placed into a syringe with a millimetre-size nozzle and is subjected to electric fields of several kilovolts. Under the applied electrostatic force, the polymer is ejected from the nozzle, whose diameter is reduced significantly as it is transported and deposited on a collector, which also serves as the ground for the electrical charges (Ramakrishna et al., 2005). The ultrafine fibre webs prepared by the electro spinning process have been extensively studied, because of their unique properties such as high surface area-to-volume ratio, small pore sizes, high porosity, and so on. In particular, the incorporation of therapeutic compounds into the electro spun nanofibres has attracted a great deal of attention, because the resultant nanofibre webs have very strong efficacy of the drug due to their high surface area-to-volume ratio, and the composite electro spun nanofibre webs afforded the prospect of preparing useful polymer systems for controlled release of activity (Kenawy et al., 2003).

Poly(vinyl alcohol) (PVA) is a synthetic water-soluble hydrophilic polymer. The basic properties of PVA are dependent on the degree of polymerisation or on the degree of hydrolysis. PVA has been intensively studied because of its good film forming, biodegradability, hydrophilicity, biocompatibility, processability, and chemical resistance (Zhang et al., 2015). It has been widely used in adhesives, emulsifiers, the textile and paper industry applications and in the attainment of amphiphilic membranes for enzyme immobilisation where properties can be improved, such as mechanical, thermal and chemical stability. Applications of PVA are limited by its hydrophilicity, but chemical cross-linking improves its stability in aqueous media. It was concluded that the heat treated electrospun PVA/$AgNO_3$ fibre web was a good material as wound dressings because it had structural stability in moisture environment as well as excellent antimicrobial ability and quick and continuous release of the effectiveness. The aim of this work was to prepare and characterise the bio cellulose impregnated with AgNPs and PVA to form nanofibres composite.

2 Materials and method

2.1 Bacterial strains

Gluconacetobacter xylinum (7795) was used in this study was received from Microbial Type Culture Collection, Chandigarh. The stock culture was maintained in the Luria-Bertani medium (LB broth). Repeated subcultures were done in LB agar plates to maintain its growth.

2.2 Culture medium

The culture was inoculated in the specific growth medium – Hestrin Schramm (HS) medium. Medium composition was as follows: D-glucose – 2%, peptone – 0.5%, yeast extract – 0.5%, citric acid – 0.115%, and disodium hydrogen phosphate – 0.11%.

Culture conditions

Incubated in two conditions such as static condition at room temperature for 20 days and shaking condition at 150 rpm for 10 days. After 10 days, the medium in the shaking condition was centrifuged at 4,000 rpm for 10 min. 0.5N NaOH was added into the pellets and incubated at 70°C for 24 hours. Centrifuged and 0.1N NaOH was added into pellets and incubated at 90°C for 15min in water bath. Filter the thin fibrous structure from the microbial disrupted cells using Whatmann no.1 filter paper. The same procedure was repeated for the medium in static condition after 20 days.

Optimisation of culture medium

To increase the yield of cellulose, the medium was optimised by using different parameters like carbon, nitrogen, growth factor, pH and temperature. Fructose, maltose, mannitol, sorbitol, dextrose, starch and D-glucose were added as a carbon source. The nitrogen sources like peptone, tryptone, sodium nitrate, ammonium sulphate and urea were used. The nitrogen source that influences the highest yield was identified and the biomass was estimated. Two growth factors, yeast extract and malt extract, were used in this study and the factor which produces higher yield was identified and the biomass was estimated. By keeping carbon, nitrogen and growth factor as constant parameters, pH was varied from 4.0–7.0. The pH that influences the higher yield was identified and biomass was estimated. In this study, the temperature between 25°C–40°C was used. The optimum temperature for the highest yield was identified and biomass was estimated. The above optimised medium was prepared and incubated in static condition at room temperature for 25 days.

2.3 Purification of bacterial cellulose

The harvested BC films were repeatedly washed with distilled water to remove some of the medium components and then boiled in 1.0% NaOH solution for 2 h to eliminate attached cells and other impurities. After that, the BC films were further purified to remove other residues by repeated washing with distilled water until the pH of the washing liquid was neutral.

2.4 Synthesis of silver nanoparticles

Fresh and healthy neem leaves are taken and rinsed with tap water and then with distilled water. A 10 g sample of neem leaves was weighed and mixed in 100 ml of distilled water and boiled for 30 min. The extract was filtered by using Whatman no 1 filter paper and stored at 4°C for further use. Different concentrations of $AgNO_3$ from 1 mM to 4 mM were prepared. To the 45 ml of $AgNO_3$ 5 ml of neem leaves extract was added. The colour changes were observed. The contact time of synthesis of silver nanoparticles was noted. The temperatures ranging from 20°C to 70°C were used to determine the influence of particular temperature for the production of silver nanoparticles. The pH of $AgNO_3$ was varied from 8 to 13 and the pH that reduces silver ion completely was determined. The morphology of synthesised silver nanoparticles was analysed by scanning electron microscope (SEM). The charge and size of synthesised silver nanoparticles were analysed by zeta potential. The synthesised nanoparticles were impregnated into the cellulose biofilm. The impregnated biocellulose and silver nanoparticles were analysed by SEM.

Fourier transform infrared (FT-IR) spectroscopy

Thin BC pellicles were evaluated through Fourier transform infrared spectra (Nicolet 6700 spectrophotometer, Thermo Scientific Inc., USA). The scan was done from 4,000 cm^{-1} to 1,000 cm^{-1} with resolution of 0.5 cm^{-1} for each measurement.

Scanning electron microscope

The BC pellicle was fixed with 2.5% glutaraldehyde solution for more than 4 h, then washed thrice in the phosphate buffer. The sample was fixed with 1% osmium tetra oxide (OsO_4) solution for 1 hour and washed thrice again. The fixed pellicle was dehydrated, using ethanol (50%, 70%, 80%, 90%, 95% and 100%) for 20 min, then transferred to the mixture of alcohol and iso-amyl acetate solution (v:v = 1:1) for 30 min, subsequently transferred to pure iso-amyl acetate for 1 h. At last, the pellicle was dehydrated in Hitachi Model HCP-2 critical point dryer with liquid CO_2, then coated with gold–palladium and observed under SEM (H-7650, Hitachi, Japan).

3 Results and discussion

The strain was cultured in LB agar and subcultured in HS agar (Figure 2) and stored in glycerol broth for further use.

Table 1 Biochemical analysis

S. no.	*Biochemical test*	*Observation*
1	Solubility test	Insoluble
2	Molisch's test	Reddish violet ring
3	Iodine test	Blue colour solution
4	Benedict's test	No change
5	Fehling's test	No change
6	Seliwanoff's test	No cherry red colour
7	Phenyl hydrazine test	No precipitates

Figure 2 Bacterial growth in LB agar plate (see online version for colours)

Figure 3 Bacterial growth on Macconkey agar plate (see online version for colours)

Figure 4 Discontinuous film of BC in the HS broth (see online version for colours)

Figure 5 Thin fibrous structure of BC

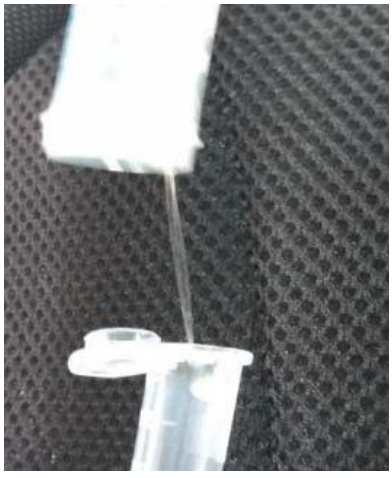


Figure 6 Image of cellulose biofilm by G. xylinus under static condition (see online version for colours)

Biochemical tests were carried out to confirm the secondary metabolite obtained from *G. xylinus* was polysaccharide. The tests are as follows – solubility test, Molisch's test, iodine test, Benedict's test, Fehling's test, Seliwanoff's test and phenyl hydrazine test. The following results were observed. The bacterial cellulose was insoluble in nature, which was confirmed by the solubility test. Reddish violet ring was observed at the bottom, which indicates the presence of polysaccharide by the Molisch`s test. Blue colour solution was obtained, which indicates the presence of polysaccharide by the iodine test. No colour change observed, which indicates absence of reducing sugar by the Benedict`s test. No cherry red colour was observed, which indicates the absence of ketose sugar by the Seliwanoff`s test. No yellow precipitate was formed, which proves that it is a polysaccharide by phenyl hydrazine test.

Figure 7 Phase contrast images of fibrous structure of biocellulose (see online version for colours)

Figure 8 Phase contrast image of *G. xylinus* (see online version for colours)

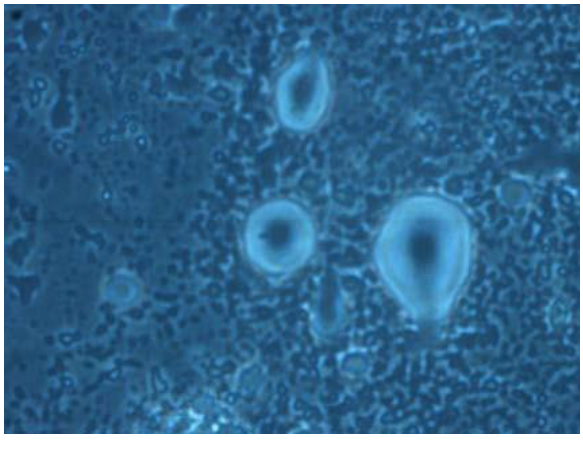


Figure 9 SEM image of crystalline *G. xylinus*

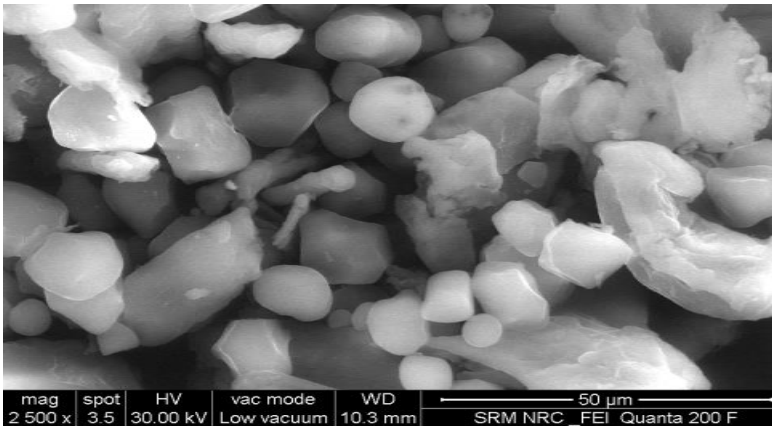


Figure 10 SEM image of bacterial cellulose

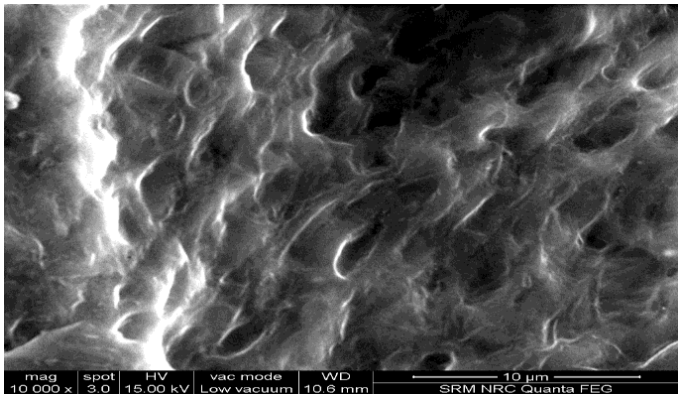


Figure 11 SEM image of Neem AgNPs

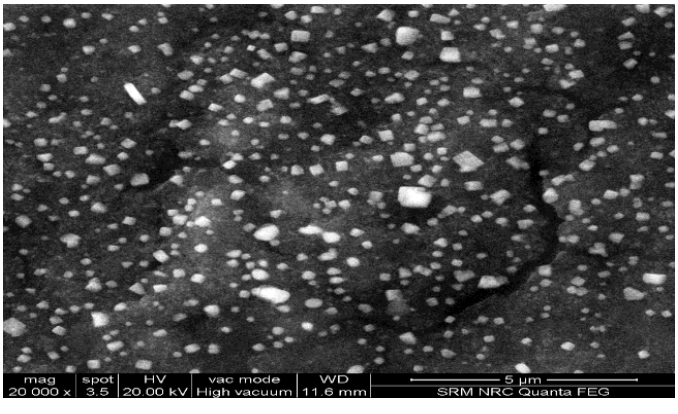


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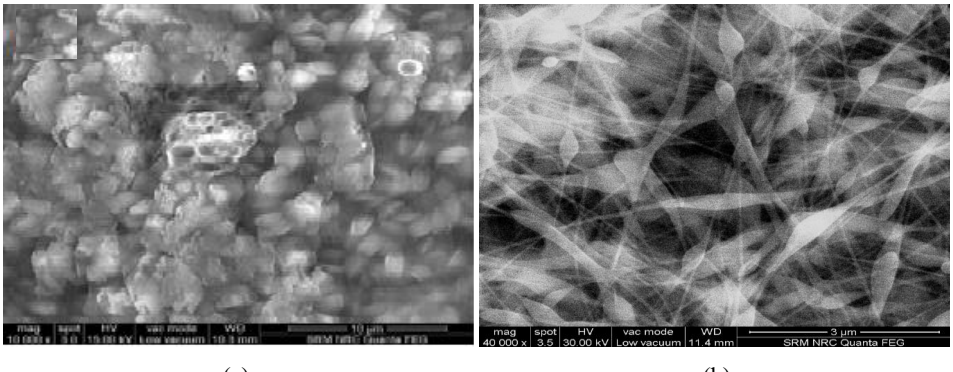
Figure 12 (a) SEM image of neem AgNPs (b) Impregnated AgNPs with bacterial cellulose



(a) (b)

The cellulose was viewed under phase contrast microscope at different magnifications. The image obtained shows an individual fibrous structure and cluster of fibrous structure (Figure 7). Individual cells observed under phase contrast microscope shows cellulose being secreted by *G.xylinus* (Figure 8).

3.1 Results for media optimisation

The cellulose was viewed under phase contrast microscope at different magnifications. The image obtained shows an individual fibrous structure and cluster of fibrous structure (Figure 7). Individual cells observed under phase contrast microscope shows cellulose being secreted by *G. xylinus.* (Figure 8). The bacterial cellulose membrane was formed in a structure with asymmetrical layers at the air/liquid interface, resulting in a denser surface, where it was in contact with air and a more gelatinous network on the other side, where it was in contact with the liquid. Similar results were obtained from various studies (Klemm et al., 2001; Backdahl et al., 2006; Camarero et al., 2004; White et al., 2004; Berti et al., 2013).

Figure 13 Effect of different carbon sources under agitation (see online version for colours)

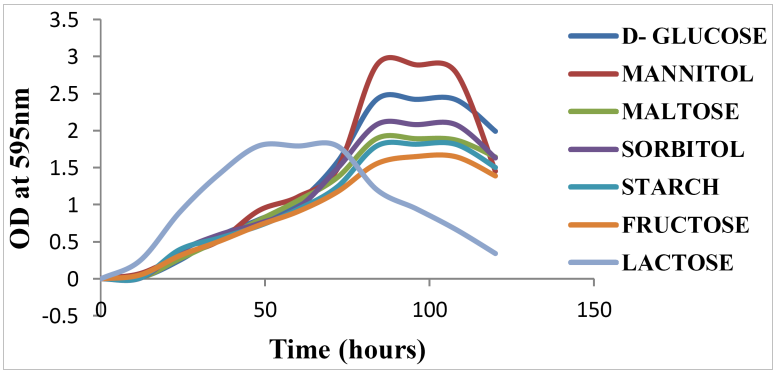


Figure 14 Effect of carbon source under shaking condition (see online version for colours)

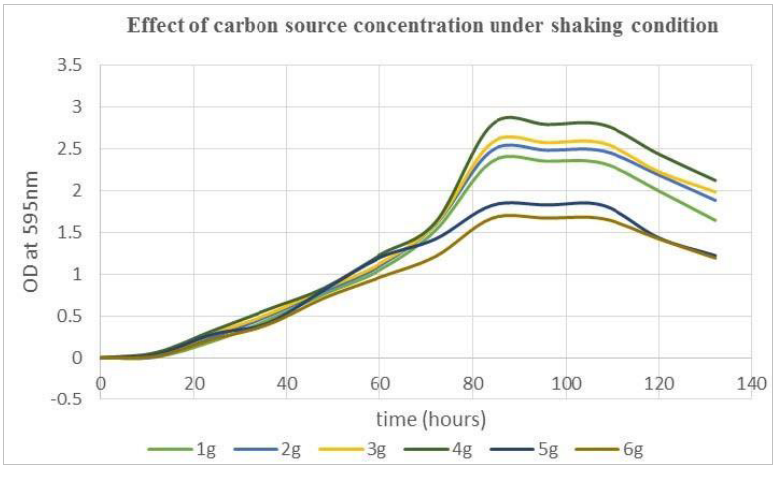


Figure 15 Effect of different nitrogen source under agitation (see online version for colours)

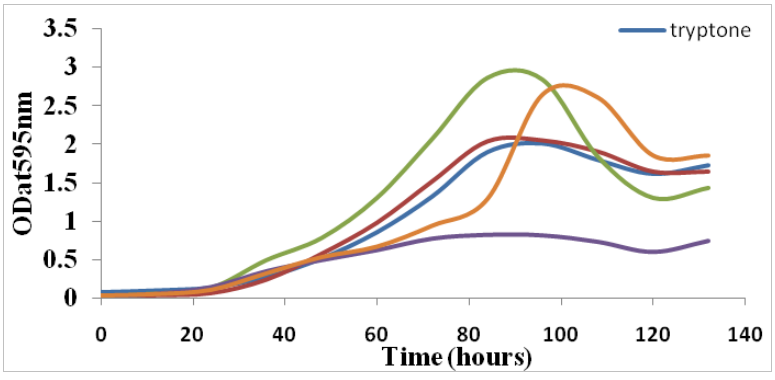


Figure 16 Effect of nitrogen source concentration under agitation (see online version for colours)

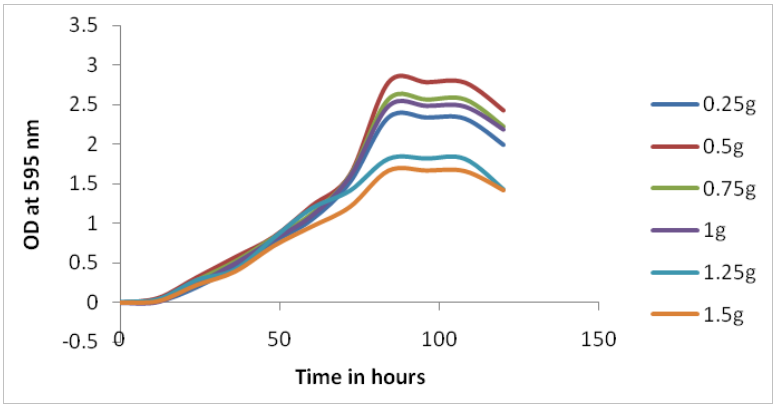


Figure 17 Effect of pH under agitation (see online version for colours)

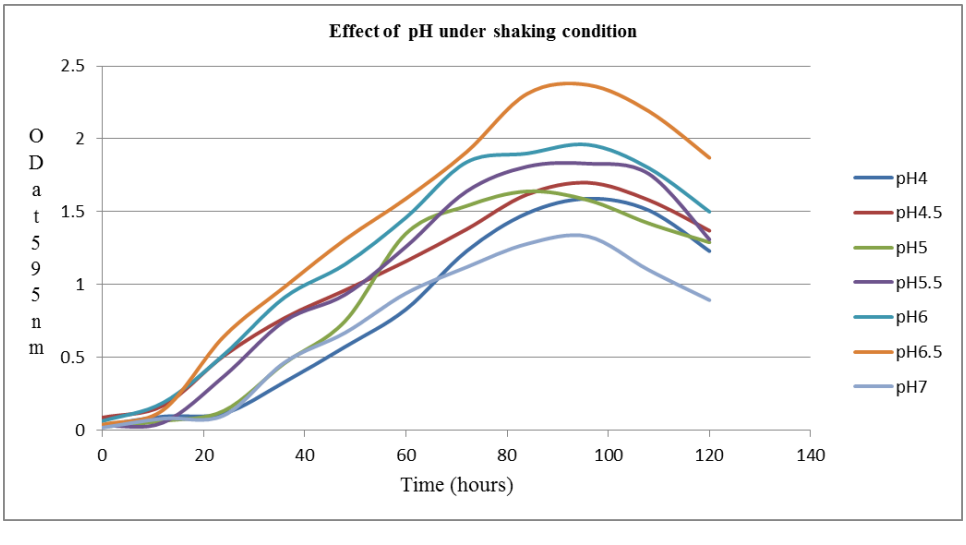


Figure 18 Effect of growth factor under agitation (see online version for colours)

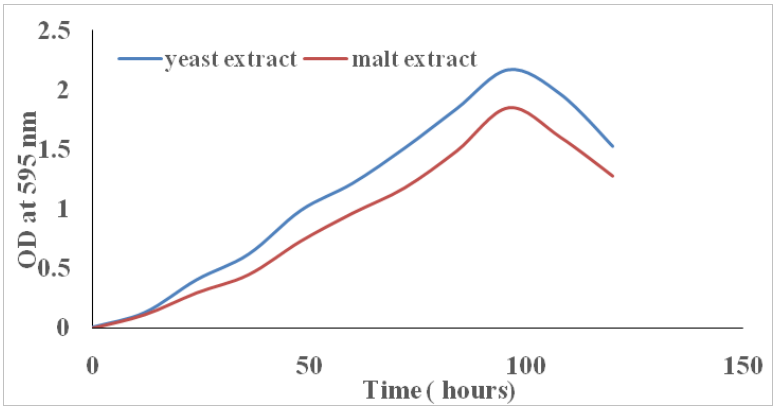


Figure 19 Effect of different carbon source under static condition (see online version for colours)

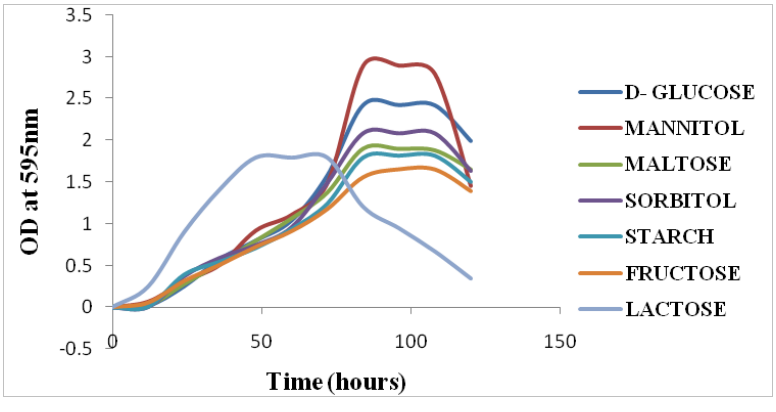


Figure 20 Effect of carbon source concentration screening under static condition (see online version for colours)

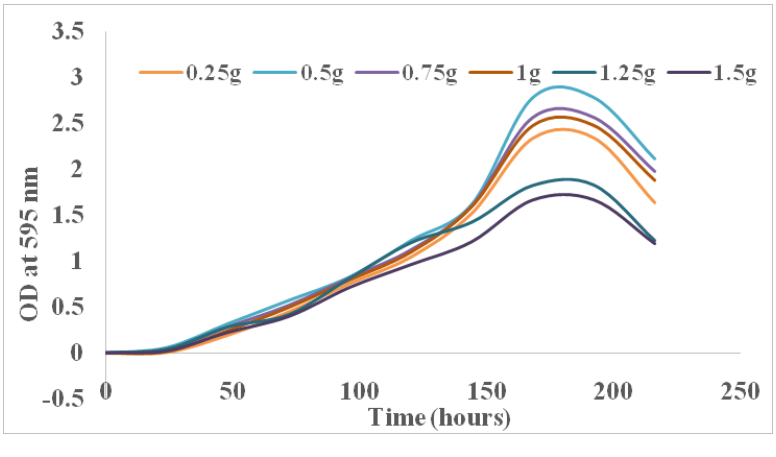


Figure 21 Effect of different nitrogen sources under static condition (see online version for colours)

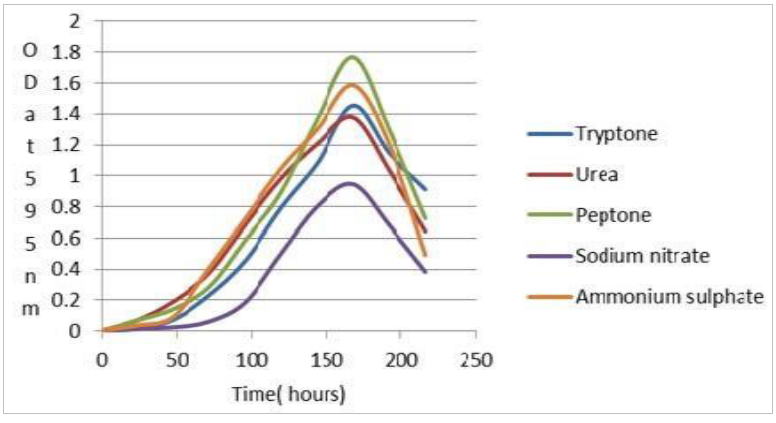


Figure 22 Effect of concentration of nitrogen source under static condition (see online version for colours)

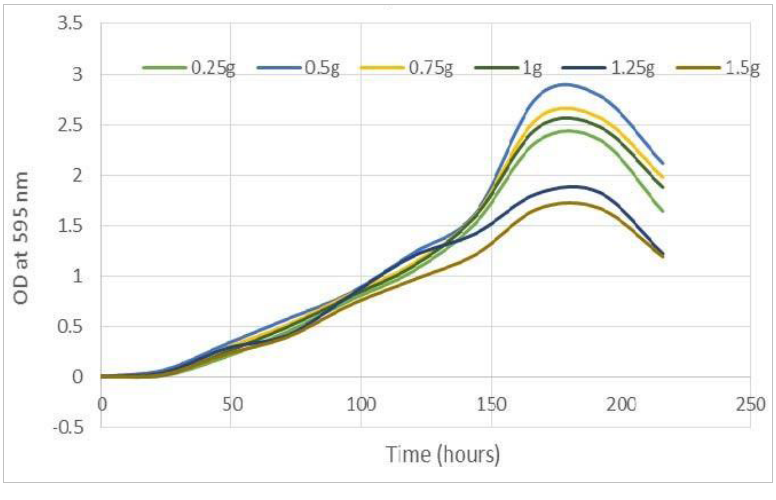


Figure 23 Effect of pH under static condition (see online version for colours)

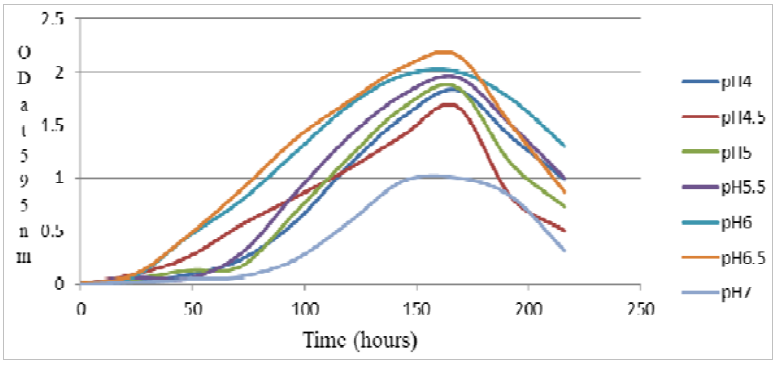


Figure 24 Effect of growth factor concentration under static condition (see online version for colours)

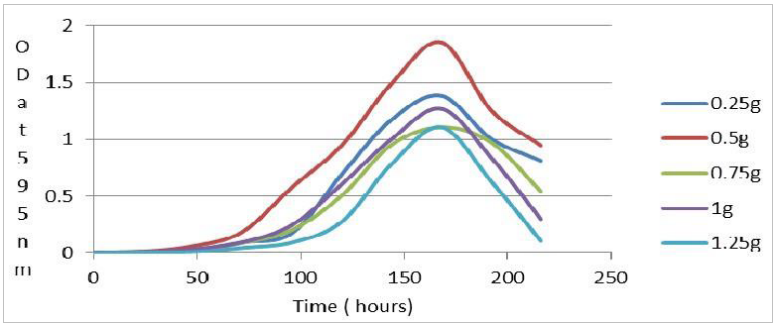


Figure 25 Biomass yield (g/L) of different carbon sources in static vs. agitation conditions (see online version for colours)

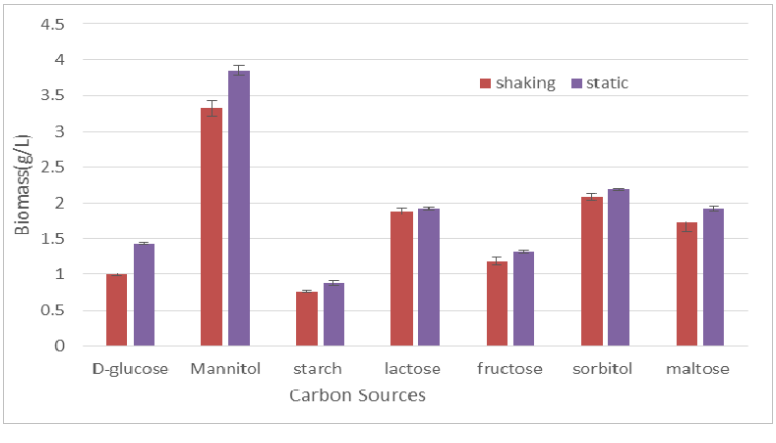


Figure 26 Biomass yield (g/L) of carbon sources in static vs. agitation conditions (see online version for colours)

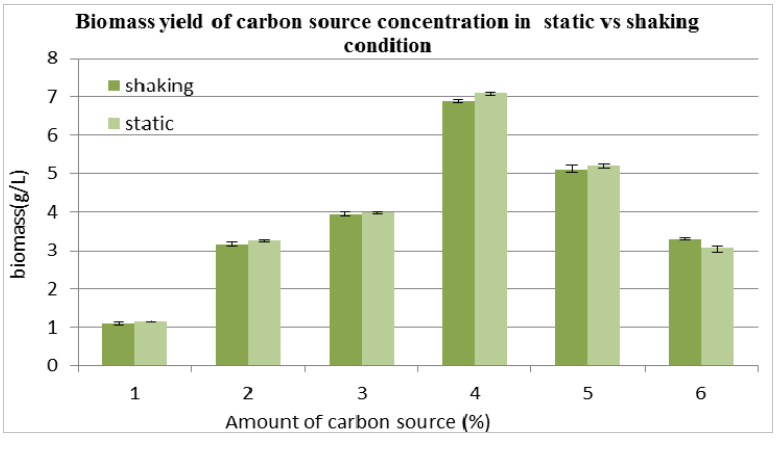


Figure 27 Biomass yield (g/L) of different nitrogen sources under static vs. agitation conditions (see online version for colours)

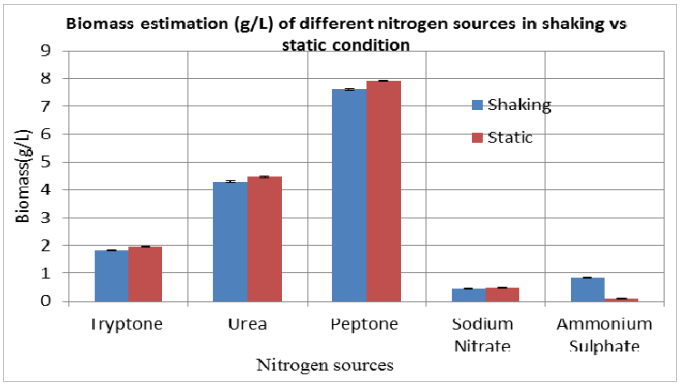


Figure 28 Biomass yield (g/L) of different nitrogen concentration under shaking vs. static condition (see online version for colours)

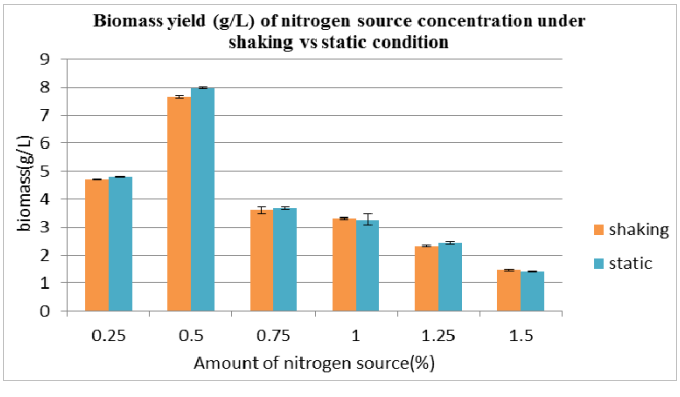


Figure 29 Biomass yield (g/L) of different pH under static and agitation conditions (see online version for colours)

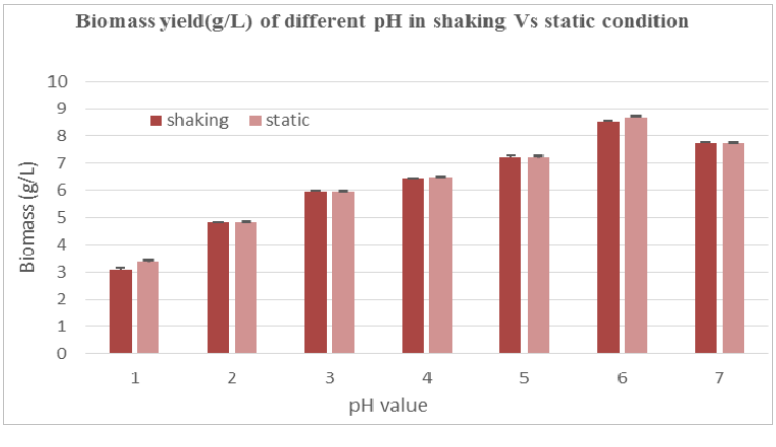
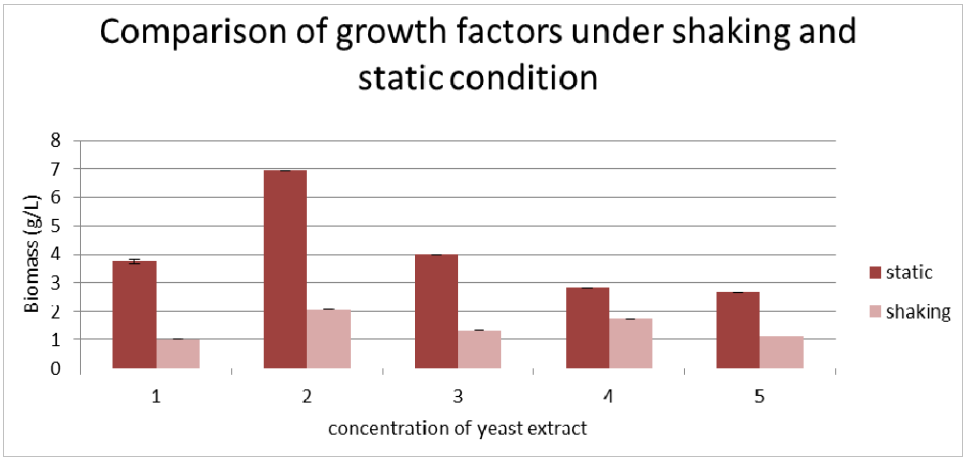


Figure 30 Biomass yield (g/L) of different concentrations of yeast under static and agitation conditions (see online version for colours)



The carbon source which influences the higher yield was identified and biomass was estimated. Different carbon sources (mannitol, D-glucose, fructose, maltose, sorbitol, starch and lactose) (Figure 13) were used to determine the optimum carbon source for the production of cellulose by *G. xylinus,* the maximum yield of cellulose was observed in mannitol, further the concentration of the carbon source was screened from 1% to 6% and maximum yield was found at 4%, hence mannitol with 4% concentration was used for further analysis. Different nitrogen sources such as tryptone, urea, peptone, sodium nitrate, ammonium sulphate were used to determine the optimum nitrogen source for production of cellulose by *G. xylinus,* the maximum yield of cellulose was observed in peptone, further the concentration of nitrogen source was screened from 0.25% to 1.5% and maximum yield was found at 0.5% and hence peptone at 0.5% concentration was used for further analysis. Effect of growth factor was analysed using two growth factors (yeast and malt extract). These growth factors were used to determine optimum growth for cellulose production using *G. xylinus,* the maximum yield was found in yeast extract, further the concentration of growth factor was screened from 0.25% to 1.25% and the optimum concentration of yeast extract was found to be 0.5%.

Different pH (4, 4.5, 5, 5.5, 6, 6.5, 7) were used to determine the optimum pH for cellulose production by *G. xylinus,* the maximum yield of cellulose was obtained in pH 6.5. Hence, the pH 6.5 was used for further analysis. Different incubation temperatures (20, 25, 30, 35, 40 and 45°C) were used to determine the optimum temperature for cellulose production by the *G. xylinus*, the maximum yield of cellulose was obtained, when the culture medium was incubated at 30 °C. At this temperature, the cellulose production reached its maximum of 8.5 g/L (Al-Shmary, 2007). All the medium components were screened under two conditions (agitation and static condition). Under shaking condition, individual cellulose pellicles were obtained due to mass transfer process. Under static condition the formation of biofilm was seen. Comparing the yield of cellulose obtained from the two conditions, it was observed that the yield of biomass under static condition was found to be more. Figures 21–24 indicate that mannitol produces 3.325 ± 0.023g/L of biomass in shaking condition and 3.855 ± 0.024 g/L of biomass in static condition. On analysing concentration of carbon source, the yield of

cellulose at 4% carbon source was found to be 6.88 ± 0.012 g/L under shaking condition and under static condition biomass yield was observed to be 7.08 ± 0.02 g/L. From Figure 25, we found that peptone gave highest yield of 7.64 ± 0.02g/L of biomass in shaking condition and under static condition the yield was observed to be 7.92 ± 0.012g/L of biomass. On analysing concentration of nitrogen source, it was observed that under shaking condition, 0.5% of nitrogen source gave 7.65 ± 0.002 g/L of biomass and under static condition 7.95 ± 0.018 g/L of biomass. In the pH study, maximum yield under static condition as well as shaking condition was obtained at pH 6.5. In the static condition, 8.66 ± 0.02g/L of biomass was estimated and under shaking condition, it was 8.05 ± 0.016g/L of biomass (Jung et al., 2005).

Figure 31 UV-VIS absorption spectrum of AgNPs AgNPs synthesised by neem leaves extract (see online version for colours)

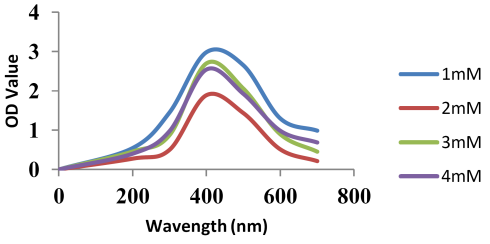


Figure 32 Effect of temperature on neem AgNP (see online version for colours)

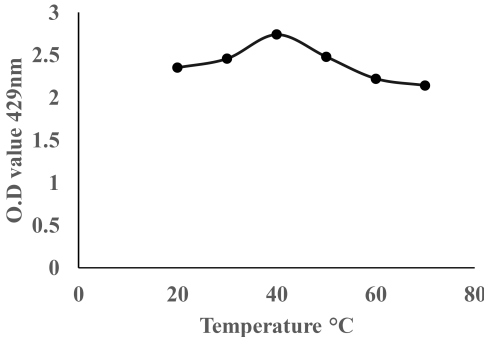
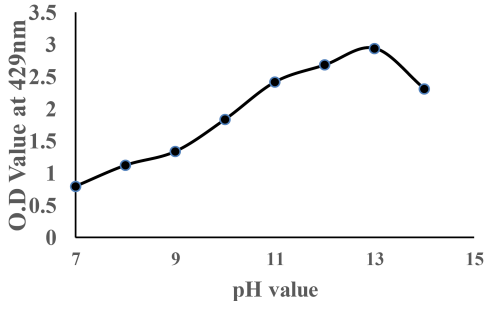


Figure 33 Effect of pH on neem silver nanoparticles (see online version for colours)



The absorption spectra of AgNPs obtained from the reaction of neem leaves extract and $AgNO_3$ were observed in the range of 300–600 nm at different reaction times. Maximum absorption was observed at 429 nm, which is a characteristic peak for AgNPs. After eight minutes, the colour of the solution containing neem AgNPs became nearly constant, indicating that no silver salt was left for further reaction (Shiv Shankar et al., 2004). With the increase in temperature (20°C to 70°C), the reduction of silver salt is enhanced, as indicated by rapid change in the colour of the solution, optimum result was obtained at 40°C (Abdelgawad et al., 2013). In the present study, upon increasing the pH from 7 to 14, the absorbance increases up to pH 13 and at pH 14 it decreases, hence maximum synthesis of nanoparticles was observed at pH 13 (Vanaja et al., 2013).

Figure 34 Effect of contact time on neem silver nanoparticles (see online version for colours)

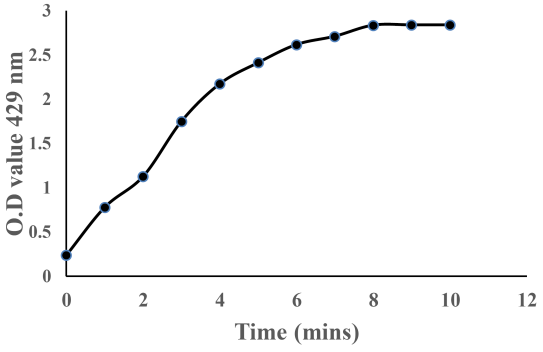


Figure 35 Zeta potential measurement of neem AgNPs (see online version for colours)

Measurement Type	:	Zeta Potential
Sample Name	:	S1 (Silver nano.)
Temperature of the Holder	:	25.0 °C
Dispersion Medium Viscosity	:	0.896 mPa·s
Conductivity	:	0.245 mS/cm
Electrode Voltage	:	3.3 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-36.4 mV	-0.000282 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

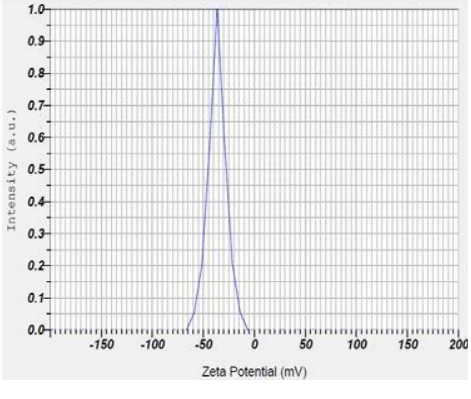
Zeta Potential (Mean) : -36.4 mV
Electrophoretic Mobility Mean : -0.000282 cm^2/Vs

Measurement Type	:	Zeta Potential
Sample Name	:	S1 (Silver nano.)
Temperature of the Holder	:	25.1 °C
Dispersion Medium Viscosity	:	0.893 mPa·s
Conductivity	:	0.245 mS/cm
Electrode Voltage	:	3.3 V

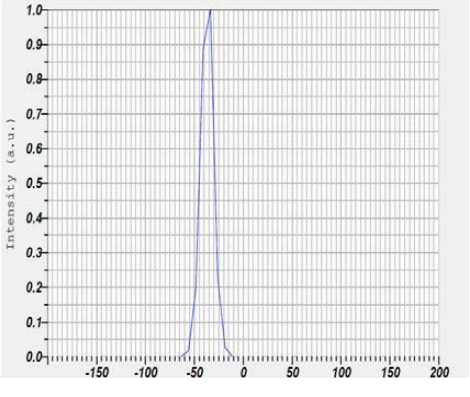
Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-37.0 mV	-0.000287 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean) : -37.0 mV
Electrophoretic Mobility Mean : -0.000287 cm^2/Vs

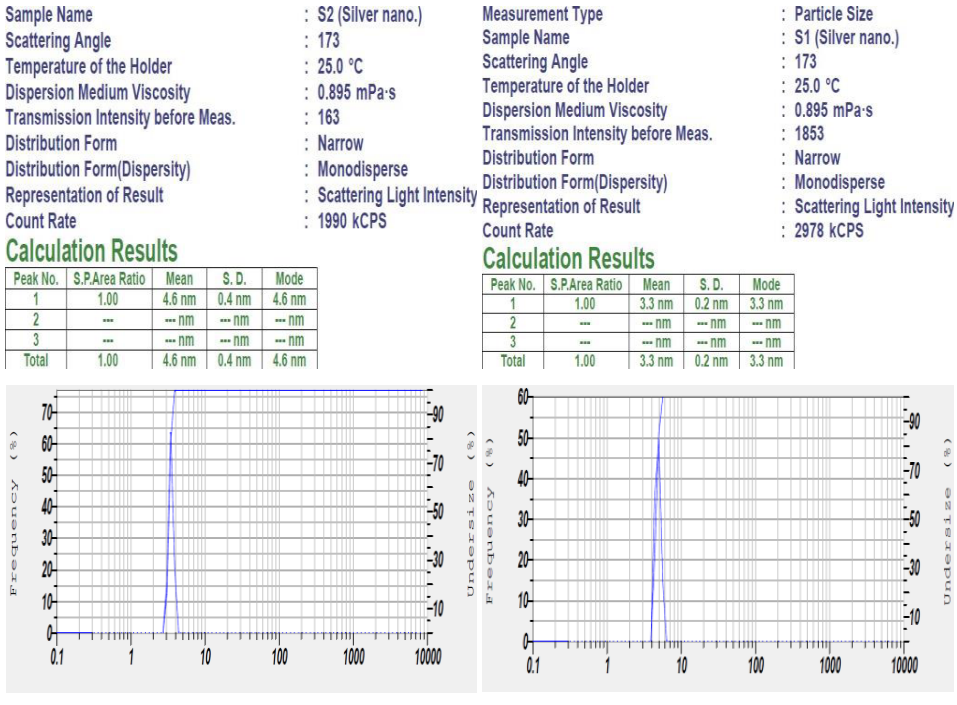


(a)



(b)

Figure 36 Particle size measurement of neem AgNPs (see online version for colours)



(a) (b)

Zeta potential measurement to know the stability of prepared nanoparticles, was observed to be –37.0 mV which indicates that it is a stabilised sample. The particle size was observed to be 3.6±0.4 nm. Also, many earlier research studies had reported that there is non-aggregation of nanoparticles in the range of ± 30 mV (Saha et al., 2010). The size measurements and zeta potentials of AgNPs indicate good stability of the synthesised AgNPs. This clearly shows that, if the hydrosol has the large negative or a positive zeta potential ≥ 30 mV, then the particles tend to repel each other and show no tendency to agglomerate, resulting in poly dispersed particles (Shiv Shankar et al., 2004).

4 Electrospinning

Electrospinning is a technique which is used widely for the electrostatic production of nanofibres. The electric power takes a part of making polymer fibres with a diameter range from nanometre to micrometre. The electrospinning apparatus consists of a source of high voltage, a spinneret, and a ground collector. The standard setup for electro spinning (ESPIN NANO) involves a syringe pump which is a 5 ml syringe, an electrical source is connected to needle of the syringe creating an electric field. The fibres are collected in the metal collecting screen. The sample were prepared in different ratios.

Table 2 Sample preparation for electrospinning

S. no.	*PVA (%)*	*Cellullose (%)*	*Neem AgNPs (%)*	*Type of flow*
1	4	2	1	Continuous
2	2	4	1	Discontinuous
3	2	2	1	Discontinuous

The fibres were formed with the flow rate at 1 ml/hr in the distance of 13 cm at 600 rpm and voltage at 20 kV. The obtained fibres were characterised by scanning electron microscopy (SEM).

Figure 37 Image of cellulose biofilm by *G. xylinus* under static condition (see online version for colours)

Figure 38 SEM image of bio cellulose film produced from *Gluconacetobacter xylinus*

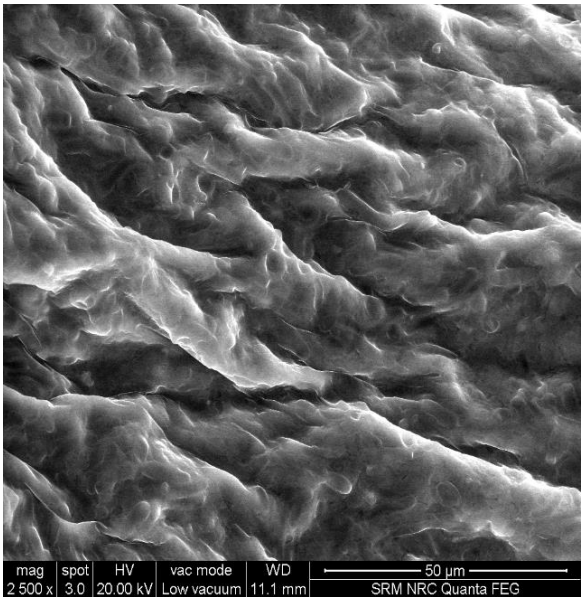


Figure 39 SEM image of porous bio cellulose biofilm

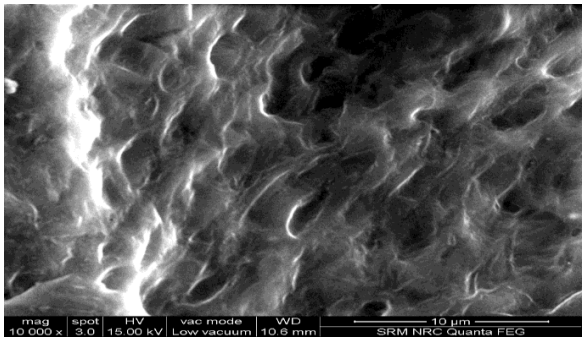


Figure 40 SEM image of neem AgNP

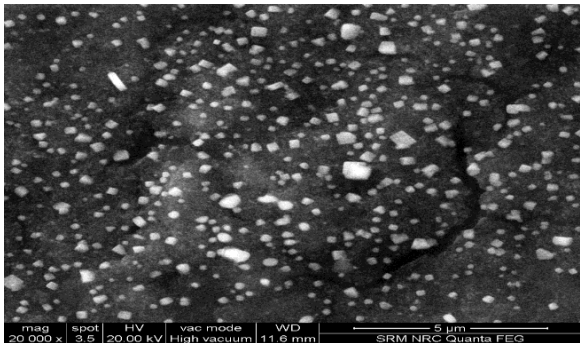
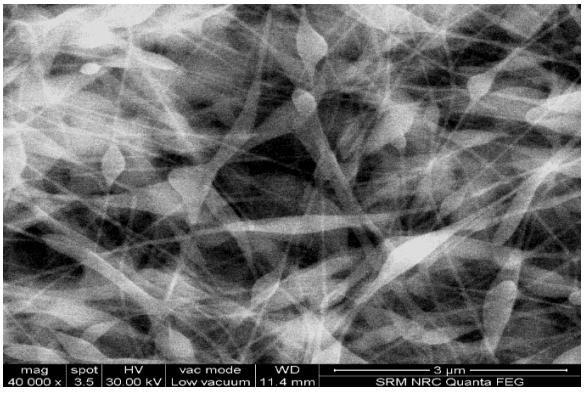


Figure 41 SEM image of electrospun bio cellulose



The FESEM image of cellulose biofilm (Figure 38) showed that the film consists of fibrous structure. On further increasing magnification (Figure 39) porous nature of biofilm was observed. The FESEM images of the silver nanoparticles are shown in Figure 40. The surface morphology of silver nanoparticles showed even size and shape. In the present study, the histogram of the particle size ranges from 20 to 50 nm (Sathishkumar et al., 2012). Nanofibres of PVA reinforced with cellulose and neem AgNPs were produced (Figure 41) by electrospinning, networks of fibres were observed

indicating the proper formation of nanofibres. The conditions for electrospinning were set as following: flow rate: 1ml/hr, distance: 13 cm, drum speed: 600 rpm, voltage: 20 kV. (Medeiros et al., 2008). The ratio of cellulose: PVA: neem AgNPs was maintained at 4%:2%:1% (Table 2).

5 Conclusions

Biosynthesis of cellulose from *G. xylinus* (MTCC 7795) using different carbon sources, nitrogen sources, and growth factors and bioprocess parameters (static vs. shaking condition, pH and temperature) have suggested significant variations in the bacterial growth rate resulting in BC yield. Structural analysis using Fourier transform infrared spectroscopy (FTIR), SEM and phase contrast microscopy proved the bacterial cellulose obtained is more crystalline than commercial cellulose under shaking condition and in static condition, biofilm was obtained. Silver nanoparticles (AgNPs) were obtained from bioreduction of silver nitrate solutions using neem leaves extract. AgNPs have been appropriately characterised using UV-vis spectroscopy, SEM and zeta potential analysis. The AgNPs from the neem leaves extract was impregnated into the biofilm obtained by *G. xylinus* using the procedure of electrospinning and by direct uptake method. The electrospun nanofibres from the bacterial cellulose, silver nanoparticles and PVA possess fibrous network, high porosity and excellent water absorption capacity. It is already known that the biosynthesised neem AgNPs have antimicrobial properties, since the biofilm is impregnated with BC, it may exhibit potential characteristics that can be used as wound healing material, because it contains biocellulose, natural neem silver nanoparticles and it is in the form electrospun nanofibres. Finally, the scale up of such biomaterial can be done with the optimum condition for the production of nanofibres with healing properties.

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