SCREENING OF MICROALGAE FOR SILVER NANOPARTICLE SYNTHESIS

Thesis submitted in fulfillment of the requirements for the Degree of

BACHELORS IN TECHNOLOGY (B.TECH), BIOTECHNOLOY

SUBMITED BY

Brinda Sharma

131570

Under the guidance of

Dr. Garlapati Vijay Kumar

Department of Biotechnology and Bioinformatics

Jaypee University of Information Technology, Waknaghat

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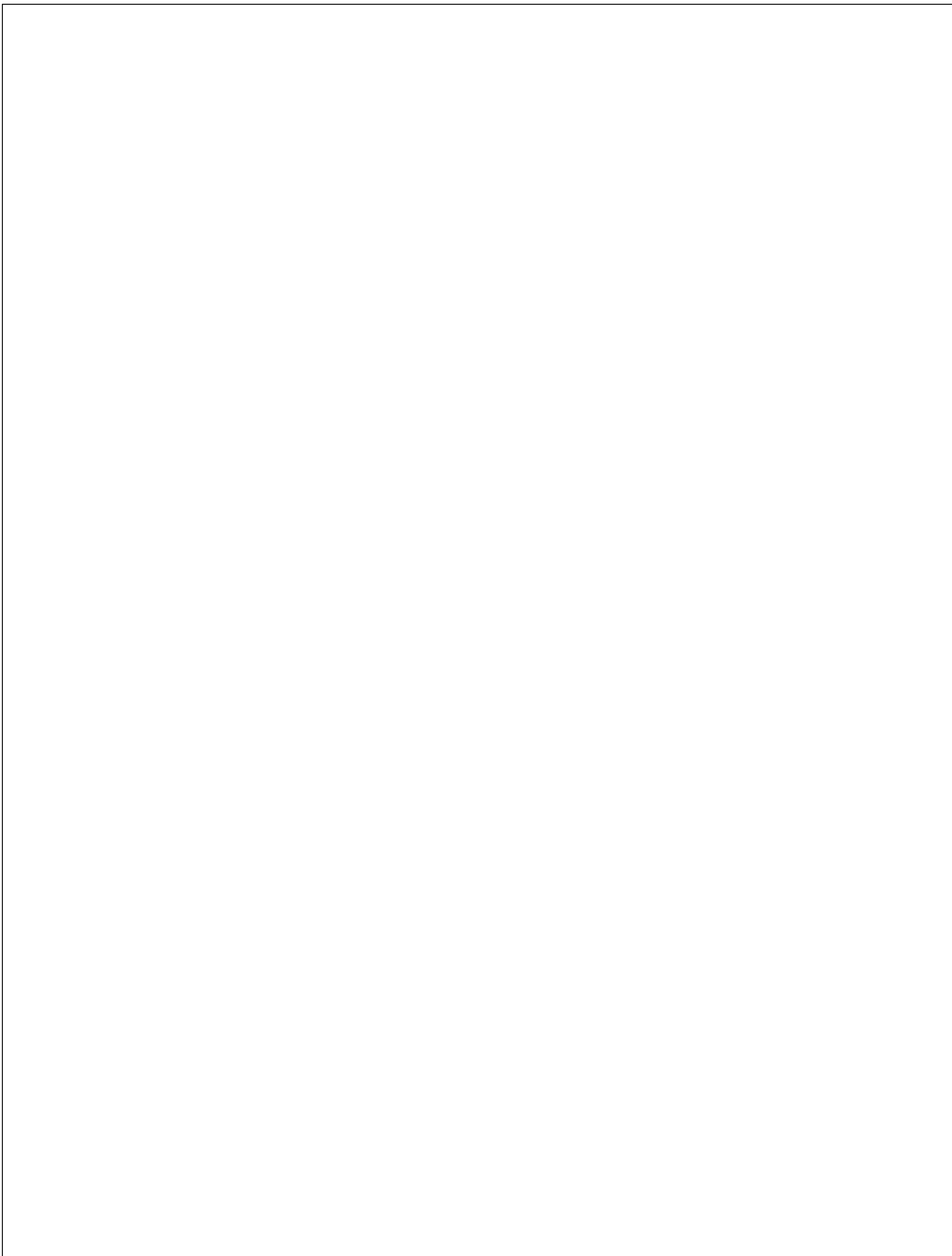

CERTIFICATE

This is to certify that the work reported in the B-Tech. thesis entitled "**Screening of microalgae for Silver Nanoparticle Synthesis**" at **Jaypee University of Information Technology, Waknaghat**, is a bonafide record of their original work carried out under my supervision. This work has not been submitted elsewhere for any other degree or diploma.

(**Dr. Garlapati Vijay Kumar**) **Date:** 01/05/2016

Dept. of Biotechnology and Bioinformatics

Jaypee University of Information Technology

Waknaghat, HP-173234, India.

Declaration

I, **Brinda Sharma, 131570** hereby declare that the work reported as B.Tech thesis entitled "**Screening of microalgae for Silver Nanoparticle Synthesis**" submitted at **Jaypee University of Information Technology, Waknaghat**, is an authentic record of my work carried out under the supervision of **Dr. Garlapati Vijay Kumar**. This work has not been submitted anywhere under any other degree.

(**Brinda Sharma**) Date: 01/05/2017

Department of Biotechnology and Bio-informatics
Jaypee University of Information Technolgy
Waknaghat, India- 173234

Acknowledgement

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(**Brinda Sharma**)

(Roll No:131570

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Abstract

Nanomaterials are a major attraction in the field of nanotechnology which is rapidly developing. Their size dependent properties make them indispensible and superior as compared to other bulk materials in a plethora of

human activities. Nanoscience gives us noteworthy approaches influencing a variety of biological processes and thus has a great impact. Especially in medicine, nanoparticles of silver (Ag-NPs) play an important role by giving already existing methodologies and procedures an edge, making them safer to use and more efficient and effective.

The aim of the experimentations was to achieve silver nanoparticle production through green synthesis using microbial cultures of microalgae by adding AgNO3 to cell culture medium as a nitrogen source. Cultures of *Scenedesmus quadricauda* were used for the same. Modification of the cultures was tried to be performed my microwave irradiation and results were compared with unmodified culture.

The result of the experiment was a colour change of the solution within 12 hours of incubation, which indicates formation of Ag-NPs or silver nanoparticles. Ultraviolet-visible (UV-vis; 300-800nm) spectroscopy was performed on samples collected every 12 hours for 5 days of incubation to obtain peaks near 370nm and 700nm which indicates different size and shape of the produced nanoparticles.

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Chapter 2

Review of Literature

Nanoscience offers discrete and sharp approaches to influence many medical and biological processes and has a great impact on medicine and biology. Nanomaterials hold shape and size dependent properties (Ahmadi et al., 1996) and their synthesis currently is a major obstacle in research even though various different processes and methods have been established. By controlling their size and their shape of the nanoparticles, their inherent properties can be controlled, depending on their application in various scientific fields (Chandran et al., 2012). Smaller particles, in this case, nanoparticles, have greater surface to volume ratio as compared to larger particles, which is vital to catalytic activity, antimicrobial properties, and thermal conductivity. These characteristics have made Nanomaterials gain applications in cancer therapy, biosensing, targeted drug delivery, disgnostic imaging, tissue engineering, anti-sense and gene therapy applications. (Chen et al., 2008). Nanomaterials have gained reputation of being multifaceted materials possessing novel or advanced characteristics compared to larger particles (Grace and Pandian 2006; Kumar et al., 2011). "Green" paths for the synthesis of nanoparticles are critical considering their integration in consumers' health and industrial products, and hence it is necessary to develop techniques to implement them. Various biological routes such as plant extracts(Shankar et al., 2004), bacteria(Pal et al., 2007), enzymes(Saifuddin et al., 2009), algae(Jena et al., 2014; Mahdieh et al., 2012; Parial et al., 2012; Rajeshkumar et al., 2014; Sudha et al., 2013; Sahayaa et al., 2012), and fungi(Vahabi et al., 2011), for environmentally sound synthesis of nanoparticles. It has been observed that microalgae can produce nanoparticles of other various metals such as gold, palladium and platinum (Brayner et al., 2007; Parial et al., 2012) besides silver. Through a diverse range of micro-organisms (Kannan and Subbalaxmi 2011) nanoparticle biosynthesis emerges via extracellular as well as intracellular pathways. Conventionally, production of nanoparticles is done by exposing micro-organisms to toxic substances. This happens either by the secretion of extracellular substances to capture the material or by electrostatic interactions (Hallmann et al., 1997). Nanoparticles can instead be formed enzymatically with either intracellular or extracellular enzymes (Mukherjee et al., 2001). Extracellularly, the reductase enzymes and electron shuttle quinines (Brayner et al., 2007) are used in the reduction of silver ions. Conversely, nutrient and substance exchange processes are imparted through intracellular formation of nanoparticles (Mahdieh et al., 2012). During intracellular formation, electrons produced by the organisms in the presence of enzymes such as reductases that are NADH dependent, reduce ions to avoid damage (Shankar et al., 2004; Kumar et al., 2007). This is suggestive of the fact that the ability of the organism to synthesize nanoparticles is determined by the metabolic status and growth phase of the organism (Gericke and Pinches 2006).

Production of silver and gold nanoparticles by reduction Au^{3+} and Ag^{+} ions using Neem (*Azadirachta indica*) was reported by Shankar et all. This is a rapid method for synthesis and requires about 2-4 hours for 90% reduction of Au3+ and Ag+ ions for Neem Leaf Broth. An example of enzymatic method of production of

nanosized particles of silver, includes using a solution of hydrazine hydrate and citrate as a reductant. Silver ions can be removed by washing thrice with de-ionised water under nitrogen stream.

Compounds derived from silver or silver based compounds like silver ions, have been known to posses antibacterial or bacteriocidal properties which has lead to an increased interest in applications of nanoparticles as antibacterial agents (Andersen, 2005). Increasingly developing Multi Drug Resistance (MDR) in bacteria has lead to a need to develop agents with antibacterial properties against both Gram Negative and Gram Positive organisms. Gram Negative bacteria posses a relatively thin cell wall which makes them more susceptible to antibacterial agents or the antibacterial effect of silver nanoparticles. Gram positive bacteria on the other hand, have a thick cell wall containing peptidoglycan layers that form a mesh, which makes them more resistant to antibacterial agents as compared to Gram Negative micro-organisms. Thus, combinatorial treatment of 'conjugating drug with nanoparticles of silver can be used for enhanced bacteriocidal effect. Due to their surface area being large, interaction and contact with microbial cells increases as compared to larger particles of the same family of materials (Hallmann et al., 1995; Mahdieh et al., 2012; Morones et al., 2005). Free radical formation that in turn induces membrane damage contributes to the bactericidal activity of these nanoparticles (Mahdieh et al., 2012).

Silver nanoparticles have also thought to be used as payload carriers of small drug molecules or larger biomolecules to specific targets. Once the silver nanoparticle-drug conjugate has reached its target, an internal or external stimulus can trigger the release of the drug. Accumulation of nanoparticles leads to high burden concentrations at the specific target which can also minimize the possible side effects.

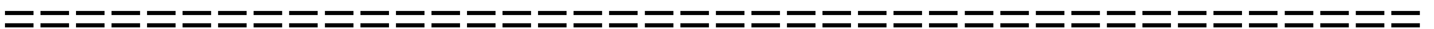
Medicine (say in fields of Diagnostic cancer imaging and drug design) is expected to be more advanced by the introduction of nanotechnology. It can help understand and give insight about function, structure organisation of the biosystem being studied, at the nanoscale. Nanoparticles can also be coated to provide a more uniform surface for substrate attachment. When the particles are coated say for example with silica, the surface has silicic acid which prevents immediate degradation by enzymes, and hence substrates can be added by stable ester and ether linkages. Recent applications have given rise to anti cancer drugs attached to a linker that is photo cleavable like an ortho-nitrobenzene bridge. This prevents degradation of this low toxicity complex, till the time required by the complex to be distributed through the body, increasing its viability. If a tumour or a cancer is being targeted, the same can be exposed or introduced to ultraviolet (UV) light, which causes the liker which is in this case photo responsive (between drug and nanoparticle) to break. The drug, unmodified, releases in the environment and acts on the target tumour or cancer. The advantage of this technique lies in the usage of nontoxic compounds for transfer to the target and the non radiative, selective release of the drug (Morones et al., 2005).

OBJECTIVES:

- Isolation and culturing of Microalgae
- Screening of Microalgae for silver nanoparticle synthesis using BG11 media
- Screening of Microalgae for silver nanoparticle synthesis using modified BG11 media and irradiated Microalgae
- Qualitative screening of synthesized silver nanoparticles through physical appearance and UV-Vis spectroscopy.

Chapter 3

Materials and Methods



3.1 Chemicals and Equipment

3.1.1 Chemicals:

- BG-11 media
- *Scenedesmus quadricauda* culture
- $AgNO_3$
- Distilled water
- $NaNO_3$
- Na_2CO_3
- EDTA
- Ferric Ammonium Citrate
- Citric Acid
- CaCl2.2H2O
- $MgSO_4.7H_2O$
- K_2HPO_4
- H_3BO_3
- $MnCl_2.4H_2O$
- $CuSO_4.5H_2O$
- $ZnSO_4.7H_2O$
- Na_2MoO_4
- $Co(NO_3)_2$

3.1.2 Materials and Equipments

Conical flasks, cotton, rubber bands, spectrophotometer, cuvettes, vials (2mL), microwave, autoclave, pipette and tips.

3.2. Media preparation

3.2.1 BG-11 media (control)

Stock solution 1	Amount (g/L)
EDTA (disodium salt)	0.01
Ferric Ammonium Citrate	0.006
Citric Acid	0.006
$CaCl_2.2H_2O$	0.036
Stock Solution 2	**Amount (g/L)**
$MgSO_4.7H_2O$	0.075
Stock Solution 3	**Amount (g/L)**
K_2HPO_4	0.04
Stock Solution 4	**Amount (g/L)**
H_3BO_3	2.86
$MnCl_2.4H_2O$	1.81
$CuSO_4.5H_2O$	0.079
$ZnSO_4.7H_2O$	0.222
Na_2MoO_4	0.39
$Co(NO_3)_2$	0.494

1. Add 1mL of stock 1, 2 and 3 and 100µL of stock 4.
2. Weigh 1.5g of $NaNO_3$ and 0.2g of Na_2CO_3

3. Add the salts and raise the volume to 1000mL (1L) with distilled water
4. Optimize the pH 0 7.5 and autoclave.

3.2.2 Modified BG-11 Media

1. For 1000mL of media, 1mL of stock 1, 2, and 3 and 100µL of stock 4 were taken.

2. 0.2g of Na_2CO_3 and 0.17g of $AgNO_3$ were weighed and dissolved with the stock measurements and volume was raised to 1000mL.

3.3 Revival and subculturing of cultures

1. Cultures had been stored in glycerol stocks after which they were transferred to BG-11 media and kept for 14 days of incubation in green house.

Experimental procedure:

1. Three flasks (500mL) were used to prepare 200mL media (for each flask).
2. For preparation of 200mL of BG-11 media, 1mL of Stock 1, 1mL of Stock 2, 1mL of Stock 3 and 100µL of Stock 4 were combined along with 0.04g of Sodium Carbonate, and 0.3g of Sodium Nitrate and the volume was raised to 200mL (For two experimental flasks, Sodium Nitrate was replaced by $AgNO_3$) and the pH was set at 7.5.
3. In Flask 1 (control), the media was inoculated with 30mL of *Scenedesmus quadricauda* culture.
4. In Flask 2, 20mL of culture was added and Flask 3 was subjected to microwave irradiation by giving 5 second pulses in the microwave 5 times with 15 second intervals between each pulse, after addition of 20mL culture.
5. All 3 flasks were placed at incubation for 2 weeks.

3.4 Setup for nanoparticles synthesis through Microalgae

300mL of reaction was set up. 300mL of normal media (containing $NaNO_3$ as nitrogen source) was inoculated with 30mL culture and kept for incubation in green house.

3.4.1 Setup for nano-particle synthesis by using modified BG-11 medium

The silver nanoparticle synthesis was carried out by two methods: (i) normal microalgae and (ii) microwave irradiated microalgae, both with 1mM $AgNO_3$ in solution as a nitrogen source.

3.5. Setup for nano-particle synthesis through irradiated microalgae by using modified BG-11 medium

In this method, the algal cultures were subjected to microwave irradiation of 5 seconds with a 15 second break, for 5 times, in a microwave.

3.5.1 Screening of microalgal cultures for synthesized silver nanoparticles

Formation of Ag-NPs (silver nanoparticles) was confirmed by UV-vis (300-800 nm) spectroscopic analysis and observed for peaks.

Chapter 4

Results and discussion

4.1 Physical observation of silver nanoparticle synthesis

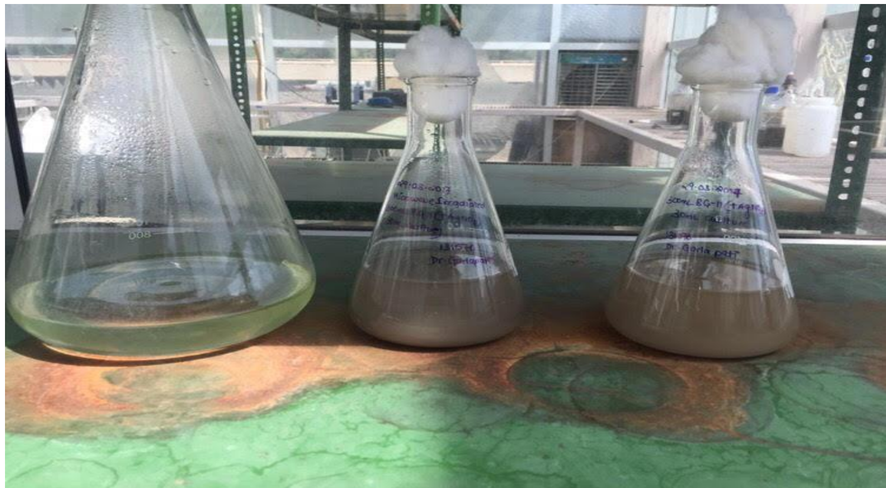


Figure 4.1: DAY ONE (from left to right: control, normal, microwave irradiated)

Figure 4.2: DAY TWO (from left to right: control, normal, microwave irradiated)

Figure 4.3: DAY THREE (from left to right: control, normal, microwave irradiated)

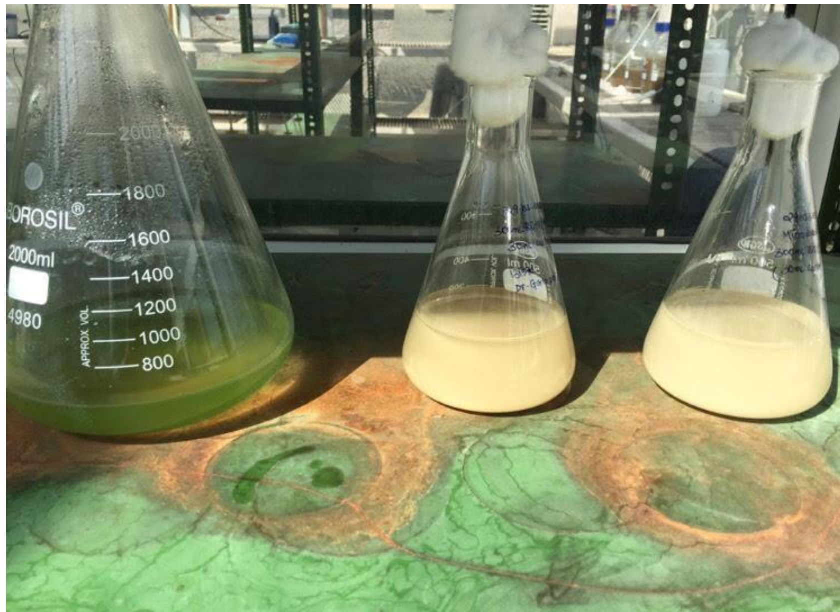


Figure 4.4: DAY FOUR (from left to right: control, normal, microwave irradiated)

Figure 4.5: DAY FIVE (from left to right: control, normal, microwave irradiated)

The formation of Ag-NPs was indicated by the change of colour of the experimental culture flasks into brown within 12 hours. The flask undergone microwave irradiation showed no visible difference as compared to the flask with normal conditions.

As incubation proceeded, the brown colour of the solutions started to fade as compared to the dark brown colour observed till 24 hours. By the end of the incubation period, brown residue collected at the bottom of the flask and the solution became relatively clear.

4.2 Screening of microalgae for silver nanoparticle synthesis through UV-Visible spectra scan

This was followed by change in UV-visible absorbance peak associated with surface Plasmon resonance of $AgNO_3$ solution.

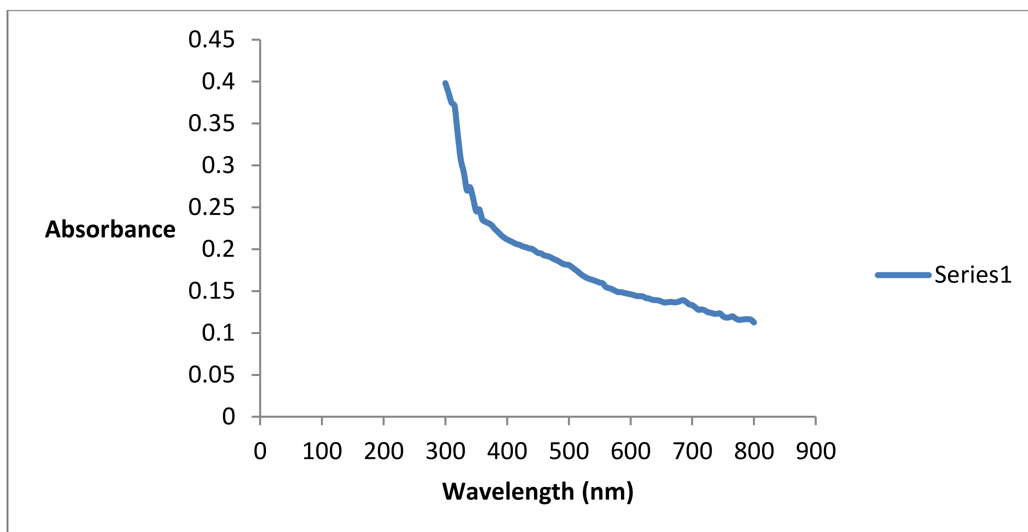


Fig 4.6. Day 5 Normal

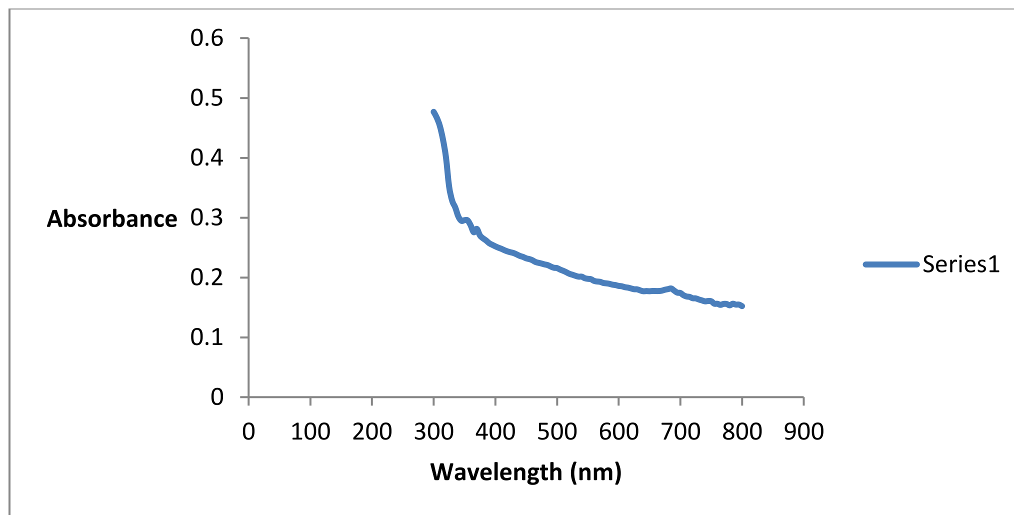


Fig 4.7. Day 5 Microwave Irradiated

Slight peaks were observed near 370nm, and 700nm. Depending on size and shape of the synthesized nanoparticles, spherical Ag-NPs having size less than 100nm give peaks near 400nm. Significant population of disc shaped Ag-NPs give absorbance around 500nm. As size of the nanoparticles increases, subsequent broadening of the plasmonic band takes places towards larger wavelengths (700nm).

Chapter 5

Conclusion

- Successful production of silver nanoparticles was indicated by the colour change of both (normal and microwave-irradiated) cultures within 12 hours of incubation
- The brown residue collection can be because of precipitation of the nanoparticles due to large size
- UV-vis spectroscopic analysis shows populations of different sized nanoparticles.
- The culture solutions contains ionic silver, and larger sized silver nanoparticles

5. Future Prospects

- The synthesized Ag-NPs (silver nanoparticles) can be separated according to their size
- Characterization can be done based on antibacterial properties of these nanoparticles.

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