Chapter 2

Experimental details of material synthesis and methods of characterization

2.1 Culture of diatoms

Diatom samples were collected from local freshwater bodies of Assam, India in the month of January 2015. 20ml of the sample was centrifuged at 3000 rpm for 15 minutes to allow sedimentation of the heavy diatom particles. The precipitate was suspended in 1 ml of distilled water and cleaned thoroughly. The collected fresh water diatoms were cultured by using "WC" medium in a BOD incubator [1, 2]. The protocol was slightly modified by lowering the pH value from 7 to 6.2 in order to make the culture media slightly acidic. The cleaned diatoms were put in conical flasks and placed in the BOD incubator for culture at 23.5ºC for 30 days maintaining 14 hours day light and 10 hours night cycle.

2.2 Description of culture process

Diatoms can be cultured basically in two ways, namely solid culture and liquid culture process [3-7]. In this work, the liquid culture process was adopted. For the liquid culture all the growth nutrients were dissolved in one liter of sterile water and the media was autoclaved at 120°C for 20 minutes. After sterilizing in the autoclave, vitamins were added to the diatom culture media in proportions tabulated in table 2.1 and then incubated with the environmental samples containing the micro-algae [8, 9]. The liquid culture was then maintained in the BOD incubator under controlled conditions of temperature 25(±0.5)ºC to 20(±0.5º)C day/night cycles; photoperiods of 16hr light (fluorescent lamps) and 8 hr dark period. At the beginning, the growth of the culture remained at the lag phase for 14 days after culture. For 21-30 days it was on the stationary phase. After the stationary phase the growth phase declined. During this culture process, cell number density increased with time and soluble silicon concentration decreased due to uptake of silicon by diatoms.

In order to prepare the WC media, the vitamins and nutrients were dissolved in 1000ml of sterile water. For this purpose, the major nutrients are prepared in 1L distilled water. After that 1ml of it was removed and 1ml of micro nutrients along with the vitamins was added to it.

Table 2.1 List of vitamins added in the diatom culture process.

Vitamin name	Amount
Thiamin HCL	0.1mg/L [T100 micropipette]
Biotin	0.5 microgm/L [T20 micropipette]
B_{12}	0.5microgm/L [29ml water+1ml B_{12}]

Table 2.2 List of major nutrients for freshwater "WC" medium.

Major nutrients (mM)	Amount(mg/L)
$CaCl_2.2H_2O$	36.76
$MgSO_4.7H_2O$	39.97
$NaHCO_3$	12.60
K_2HPO_4	8.71
$NaNO_3$	85.10
$Na_2SiO_3.9H_2O$	56.84

Table 2.3 List of micronutrients for freshwater "WC" medium.

Micronutrients (μM)	Amount (mg/L)
Na_2EDTA	4.36
$FeCl_3.6H_2O$	3.15
$CuSO_4.5H_2O$	0.01
$ZnSO_4.7H_2O$	0.022
$CaCl_2.6H_2O$	0.01
$MnCl_2.4H_2O$	0.18
$Na_2MoO_4.2H_2O$	0.006
H_3BO_3	1.0

2.3 Preparation of samples for chemical and structural characterization

In order to characterize and analyze the (untreated and As-treated) diatom frustules we need a proper cleaning procedure to remove the external organic part that cover the frustules and mineral debris (mud, sand, slit, leaves of aquatic plants, harvest from planktons, etc.). Normally acid treatment method is used for cleaning of diatom frustules [2]. However for better results some modification was done in the cleaning process using both acid and base treatment. This was done by the following procedure. A block diagram for the diatom culture process is shown in figure 2.1.

1. The culture flask was shaken for 5 minutes to detach all diatoms.
2. The pellet was washed in double distilled water (DW) several times to remove the excess of fixative.

3. H_2O_2 was added to the samples and heated for 12 hours in the water bath at 60 ºC to remove mineral debris.
4. Removal of H_2O_2 was done by centrifuging the sample at 3000rpm for 10 minutes by adding DW and the process was repeated several times.
5. 37% aqueous HCl was added to the sample and put aside for two hours. After that the sample was cleaned with DW by using sedimentation method and then DW was pipetted out.
6. The cleaned frustules were then stored in ethanol to avoid contamination and bacteria growth.
7. For structural analysis using SEM, about 20µL of suspension in methanol was pipetted drop wise onto rectangular/circular cover slips and then dried in air.
8. For FTIR analysis, 2.0mg of frustules were mixed with 100mg KBr and ground to fine powder and 50mg of this mixture was compressed for 5minutes by a hydraulic hot press to make a transparent pellet which was mounted onto the FT-IR sample holder for further analysis.

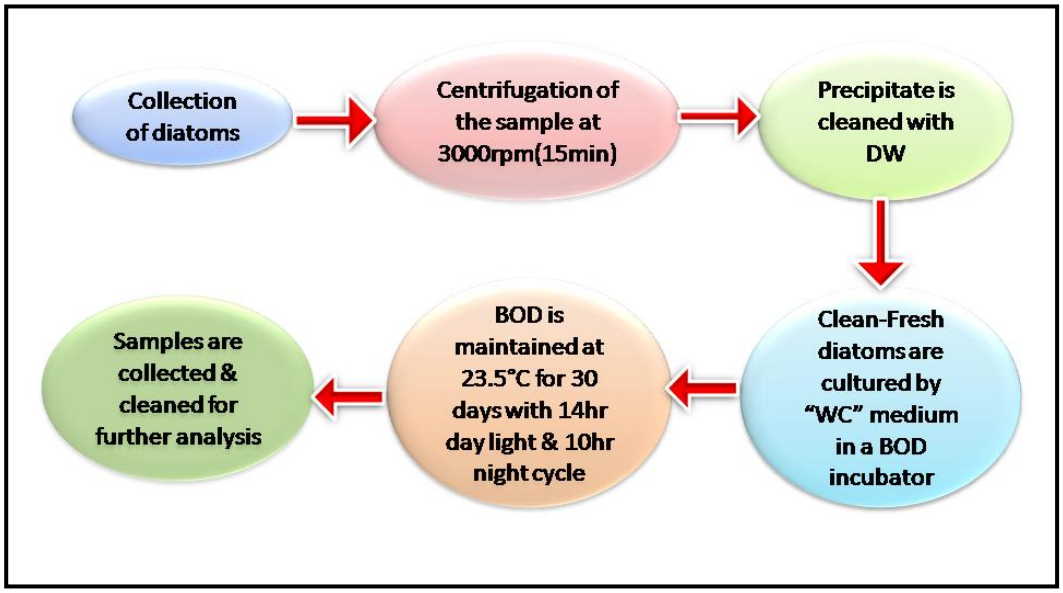

Figure 2.1 Block diagram of diatom culture process.

2.4 Method of synthesis of DT500 and DT800

Diatoms species were collected from a local fresh water natural lake called Padumpukhuri, located at Tezpur, a city of the state Assam in India. The collected diatoms were cultured by using WC media [1, 2]. After 30 days the cultured diatoms were cleaned by distilled water and then kept in 4% glutaraldehyde (GA) solution for 12 hours. After the GA treatment, the diatoms were again cleaned with distilled water. Thereafter they were immersed in 2M HCl (35%) for another 12 hours. These diatom templates were then dehydrated and preserved in ethanol. In the next stage, the ethanol solvent was replaced gradually by isopropanol. Again, 5% titanium tetraisopropoxide (TTIP) was separately prepared with isopropanol. The as-prepared templates were stirred slowly while also adding TTIP in a conical flask to carry out the titania nanostructure coating process for 24 hours. Next, frustule samples were filtered out from the solution and were air dried. These samples were then calcined at 500^{o}C and 800^{o}C in air for the transformation of phases [10, 11] to prepare DT500 and DT800 sample respectively. Finally these diatom frustule templated titania nanostructures were used as photocatalyst in our research work.

2.5 Procedure of photocatalytic activity of DT500 and DT800 catalyst

In order to study the photocatalytic behavior of the as-prepared diatom frustule templated titania nanostructure sample, examination was done on an aqueous solution of MO prepared in the following manner - 30mg of each of prepared photocatalysts (DT500, DT800) were added to 100ml of 10μM MO solution and stirred in the dark for 10 hours so that the system reached adsorption-desorption equilibrium. Thereafter the solutions were exposed to visible-light irradiation. After every half an hour, 8ml of this MO suspension was taken out and then centrifuged for 12 minutes at 6000rpm to precipitate out the catalyst particles from the solution. From the upper portion of this solution 3ml of the aliquot was pipetted out, avoiding the precipitate, for studying the extent of photodegradation process.

Photocatalytic degradation of MB was also done by adding 20mg of prepared photocatalyst (DT500 and DT800) in 100ml of 20μM and 50μM MB solution and stirred in the dark for 3hours. There after the solution mixture was exposed to visible-light irradiation. After every 10 minutes during the photodegradation process, 10ml of MB suspension was taken out and centrifuged for 10 minutes at 7000rpm to remove the catalyst particles from the dye solution. From this 3ml of the aliquot was taken for further analysis.

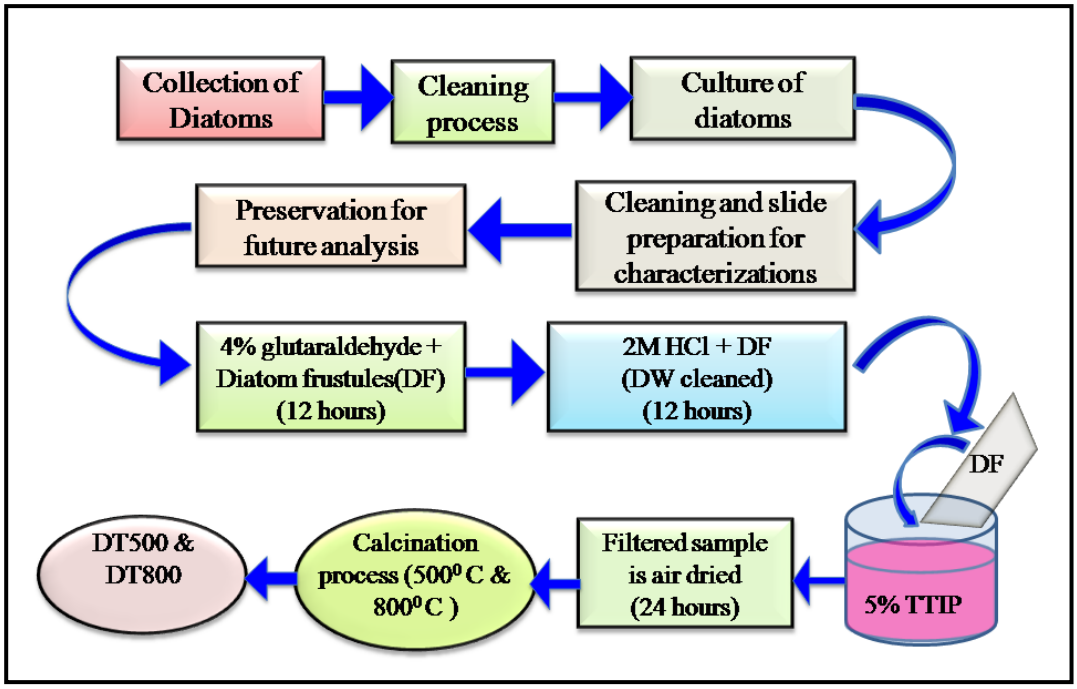

Figure 2.2 Block diagram of synthesis of DT500 and DT800 samples.

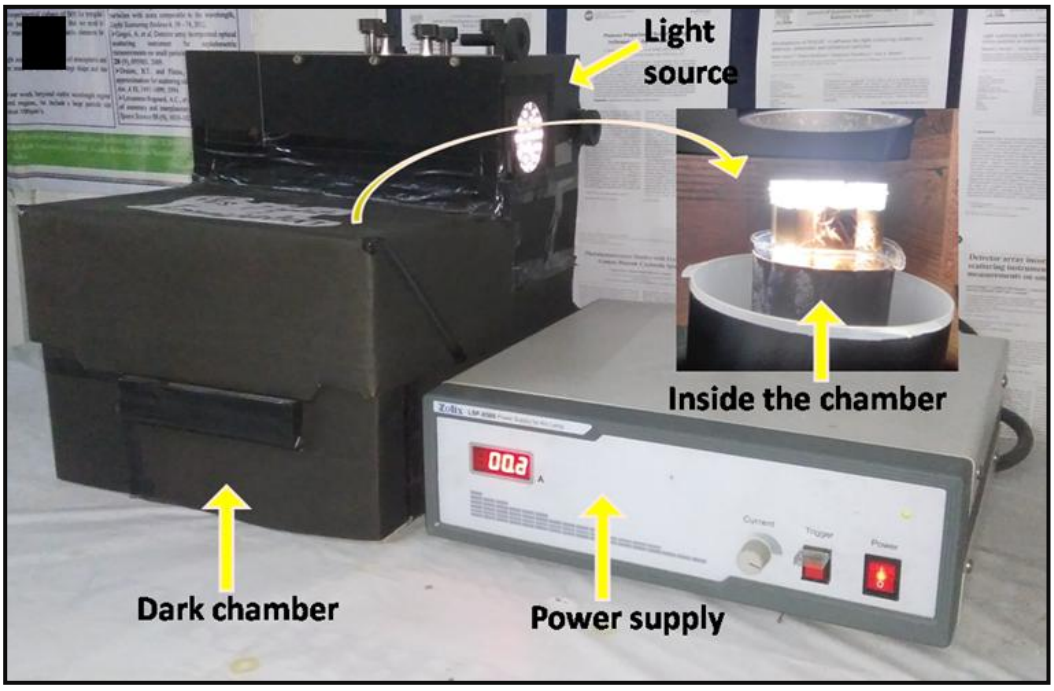

Figure 2.3 Image of experimental photocatalytic measurement set-up.

2.6 Details of green synthesis of silver nanoparticles using diatom frustules

The green synthesis of Ag-NPs using diatom frustules as template is explained in details in this section. Diatom species *Navicula* was collected from local fresh water bodies of Assam, India in the month of January. The sample was cleaned with DW. The collected fresh water diatoms were cultured by using "WC" medium in a BOD incubator [1,2]. The protocol was slightly modified from the existing reports by lowering the pH from 7 to 6.2 in order to make the culture media slightly acidic. The cleaned diatoms were put in a conical flask and kept in the BOD incubator for culture at 23.5ºC for 30 days maintaining 14 hours day light and 10 hours night cycle. This species was cultured in WC media for 30 days. After the culture process, diatoms were cleaned by DW followed by sedimentation and then pipetted out. In order to isolate diatom frustules, we need a proper cleaning procedure to remove external organic part that covers the frustules and mineral debris (mud, sand, slit, leaves of aquatic plants, harvest from planktons, etc.). In this work, this was done by using the following procedure:

(a) Diatom cells were cleaned with distilled water (DW),

(b) Sedimented diatoms were cleaned by H_2O_2 followed by HCl for few minutes and then cleaned with DW again.

These precisely cleaned diatom frustules were used for the preparation of Ag-NPs in presence of light. For this purpose, 10mM $AgNO_3$ solution was prepared in a conical flask and the cleaned frustules were added in this solution in presence of light to initiate the reaction at room temperature. In order to ascertain that light is a necessary factor for the Ag-NPs preparation using this method, another set of experiments was performed in which diatom frustules contained $AgNO_3$ solution was kept in the dark. In the first experiment, it was observed that after few hours the color of the solution gradually changed from colorless $AgNO_3$ solution to light purple color which confirmed the formation of NPs (figure 1) [12-17]. Finally the color of the solution became dark purple and the dark purple colored frustules were sedimented in the conical flask and kept in the dark so that no other light induced effect could occur and used for further analysis. The Ag-NPs templated on diatom frustules were named as SNDs.

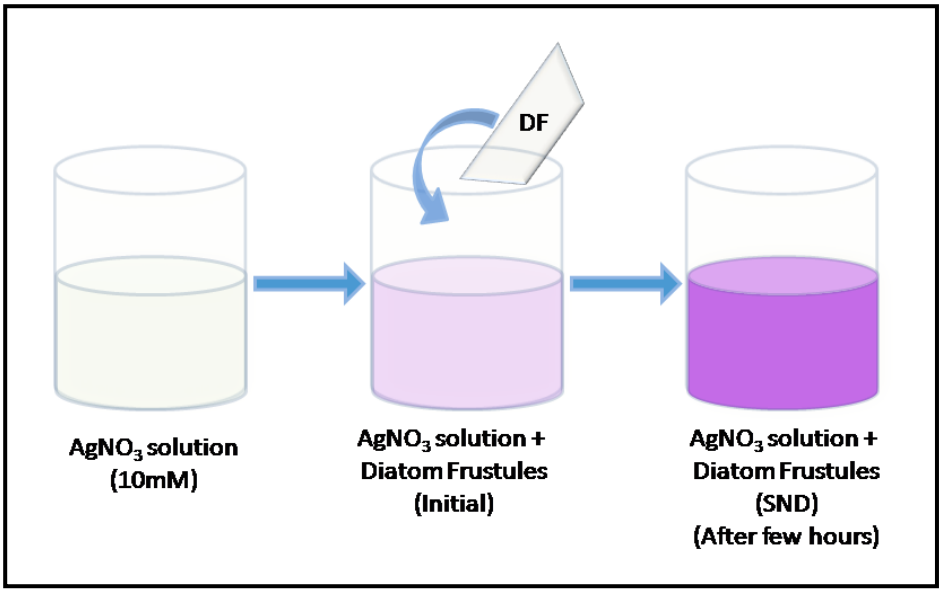

Figure 2.4 Schematic diagram of SND synthesis process.

2.6.1 SND sample preparation for chemical and structural characterization

For SEM and XRD analysis, sufficient amount of suspension (SND sample) was pipetted onto rectangular/circular cover slips and then dried in the dark. For FTIR analysis, 2.0mg of SND sample was mixed with 100mg KBr and ground to fine powder and 50mg of this mixture was compressed for 10minutes by a Hydraulic Hot Press to make a transparent pellet which was mounted onto the FT-IR sample holder. It was observed that there was no change in color of the solution in case of the second experiment revealing that light is a necessary factor for the formation of Ag-NPs using diatom frustules.

2.6.2 Technical procedure for ammonia sensing using SND sample

The sensing of ammonia was initially visualized by the change in color with the addition of 0.5ml of as prepared SND sample in ammonia solution having different concentration ranging from 0ppm to 100ppm. It was observed that the color of the solution changes gradually from purple to light yellow with the increase in ammonia concentration. Figure1 envisions the color change during addition of SNDs in the ammonia solution. Moreover, the optical sensing of ammonia using SND sample with the help of UV-vis absorption spectra analysis will be illustrated in section 3.3 of chapter 3.

2.7 Experimental details

2.7.1 Characterization of arsenic treated and untreated diatom frustules

A detailed analysis of the morphology of arsenic treated and untreated diatom frustules was done by JEOL JSM-6390LV scanning electron microscope and INCAx-sight energy dispersive X-ray spectroscopy (EDS) detector. Optical microscope (make: Axiostar Plus optical microscope with 100x resolution) was also used to study the morphology of the frustules and classify the microbiological organisms. The XRD patterns of untreated and arsenic treated diatom samples were collected using a RIGAKU MINIFLEX diffractometer with Cu-Kα radiation (λ=1.5405 Å). FT-IR measurements were recorded after averaging 32 scans with 2.0 cm^{-1} resolution on a FT-IR instrument (make: Nicolet Instruments 410) equipped with KBr optics and DTGS detector. The optical absorption spectra of diatom samples were obtained from a UV visible absorption spectrophotometer (make: UV 2450, Shimadzu Corporation).

2.7.2 Characterization details of DT500 and DT800

The morphology and classification of the microbiological organisms was done using a Scanning electron microscope (SEM) (JEOL JSM-6390LV). FT-IR measurements were taken with an average scan rate of 32 scans and with 2.0 cm^{-1} resolution in a FT-IR spectrometer (NICOLET INSTRUMENTS 410) equipped with KBr optics and pyroelectric DTGS (Deuterated Tri Glycine Sulfate) detector. 2mg of prepared samples were mixed with 100mg KBr and ground to fine powder and 50mg of this mixture was compressed for 5min by a Hydraulic Hot Press to make a transparent pellet which was mounted onto the FT-IR spectrometer sample holder for analysis. XRD patterns of the samples were obtained using a x-ray diffractometer (RIGAKU MINIFLEX) with Cu-Kα radiation line (λ=1.5405 Å) for structural analysis. Full scans (2θ, 20º-80º) were performed with a step size of 0.05º and scan rate of 1.0 2θ/min. The optical absorption spectra of these diatom frustule templated titania nanostructures, namly DT500 and DT800, were obtained using a UV-visible absorption spectrophotometer (UV 2450, Shimadzu Corporation). Next, the as-prepared samples were dispersed in ethanol and ultra-sonicated for 3 hours and then they were drop casted on a copper grid for analysis using a Transmission Electron Microscope or TEM (TECNAI G2 20 S-TWIN, 200KV, FEI USA) so as to obtain structural phase information of the samples. Finally, for information on the structural modes, a Raman spectrometer (E Z RAMAN-N), with an excitation wavelength of 785nm, was used. Pore size distributions and BET surface area were measured by N_2 adsorption/desorption isotherm using a NOVA 1000E BET instrument (make: QUANTA CHROME). Prior to this analysis the samples were degassed at 150 ºC for one hour.

Photocatalytic degradation of MO with the as-prepared catalysts was carried out under 500W visible light irradiation from a Xenon Arc Lamp source (Zolix SLH- X500) and the degradation process was monitored at regular intervals with the UV-visible absorption spectrometer.

2.7.3 Characterization of as-synthesized silver nanoparticles

The morphology and classification of the microbiological organisms and deposition of silver nanoparticles on diatom frustules, namely SND samples, were studied using a Scanning electron microscope (SEM) (JEOL JSM-6390LV). XRD patterns of the samples were obtained using a x-ray diffractometer (RIGAKU MINIFLEX) with Cu-Kα radiation line (λ=1.5405 Å) for structural analysis. Full scans (2θ, 20º- 80º) were performed with a step size of 0.05º and scan rate of 1.0

2θ/min in the XRD. The optical absorption spectra of these materials were obtained using a UV-visible absorption spectrometer (UV 2450, Shimadzu Corporation). FTIR measurements were taken with an average scan rate of 32 scans and with 2.0 cm^{-1} resolution in a FT-IR spectrometer (NICOLET INSTRUMENTS 410) equipped with KBr optics and pyroelectric DTGS (Deuterated Tri Glycine Sulfate) detector.

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