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Characterization of silica nanoporous structures of freshwater diatom frustules

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ABSTRACT:

A phytoplanktonic unicellular alga known as diatoms belonging to the class Bacillariophyceae, possess a distinct, highly ornamented siliceous cell wall consisting of two overlapping halves. Diatoms are found both in marine and freshwater environment and also in moist habitats. A study was designed to assess and examine the morphology of diatoms in *Chapanala* and *Jiajuri*, two silica rich sites in Nagaon district of Assam as reported by Geological Survey of India. Samples were collected from aquatic and semi-aquatic habitats of the study sites and immediately transferred to Diatom specific Media. The samples were then subjected to acid wash treatment for detailed microscopic observations. Nanoporous structures of freshwater diatom frustules have been well characterized through extensive SEM analysis. The prominent forms include - *Pinnularia* sp., *Navicula* sp., *Achnanthidium* sp., *Nitzschia* sp. and *Eunotia* sp. The SEM micrographs very clearly showed the presence of fine nanostructure pores, the valve view and distinct raphe of the diatoms. In the present study, the sizes of nanoporous silica were found in the range of ~60-170 nm under SEM observations, suggesting the potentiality to use the diatoms in various nanotechnological applications.

Keywords:
Freshwater diatom, Frustule, Silica, SEM, Geological Survey of India.

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INTRODUCTION

Diatoms areeukaryotic, unicellular or colonial microalgae inhabiting a wide variety of habitats. Diatoms are microscopic, sizes ranging from 2µm to 2mm and species are classified mostly by the shapes and patterns of their hard silica parts. The most characteristic feature of diatoms is their cell wall or exoskeleton which is built up of amorphous silica. These extremely diverse group of phytoplankton form the basis of many aquatic food chains, and are thought to be responsible for upto 25% of the world's net primary productivity. The frustules possess intricate nanoscale features such as pores, ridges, areoles, spikes and spines imbedded within the periodic two-dimensional pore arrays. They are the only organisms known to possess genetic ability to mineralize amorphous silica into complex structures. Diatoms are particularly attractive for nanotechnology because they build their highly symmetric skeletons with a nanopattern directly in 3D form (Round *et al.,*1990). Biomineralize silica cell walls confer the diatoms diverse and impressive exoskeletal architecture (Montsant *et al.,* 2005; Bozarth *et al*., 2009). The diversity of the silica structures on the diatom cell walls appears to be quite significant and extends possibilities for their use in nano-fabrication of a multitude of devices having wide ranging applications in biochemical analyses, microsensors, computing and telecommunications, optical devices, microrobotics, micro batteries etc. (Gordon and Parkinson, 2005).

Silica sand deposits have been reported by the Geological Survey of India (GSI) in the *Jiajuri* and *Chapanala* region of Nagaon district of Assam (Borpuzari, 2012). *Jiajuri* hill (26° 18' 0'' to 26° 19' 0'' N latitude and 92° 52' 55'' to 92° 54' 15'' E longitude) covers an area of 2.9 km^2 and the possible friable quartzite is about 7.4 million tones. The friable quartzite deposits of *Jiajuri* occurs on plateau with undulating topography. Chapanala is bounded by latitude 26° 20' 10'' N and longitude 92° 51' 30'' E, covering an area of 0.373 km^2 and possible reserve is 3.5 million tones (Borgohain and Tanti, 2014). No any extensive investigation has been carried out to characterize the diatom from these silica rich areas.

MATERIALS AND METHODS

Cell collection and culture

Water and semi-aquatic soil samples were collected from the sampling sites, *Chapanala* and *Jiajuri* on the basis of habitat stratification (Fig.1). The collected samples were then transferred in the DM (Diatom Medium) proposed by Beakes *et al*., (1988). The medium was standardized with slight modification and the composition of stock (per 200ml) includes- $Ca(NO_3)_2.4H_2O$ – 4g, KH_2PO_4– 2.48 g, $MgSO_4.7H_2O$ - 5 g, $NaHCO_3$ – 3.18 g, EDTAFeNa – 0.45g, $EDTANa_2$ – 0.45g, H_3BO_3 – 0.496g, $MnCl_2.4H_2O$ – 0.278g, $(NH_4)6Mo_7O_{24}.4H_2O$ – 0.20g, Cyanocobalamine - 0.008g, Thiamine HCl – 0.008g, Biotin – 0.008g and $Na_2SiO_3.9H_2O$ – 22.8g (Borgohain and Tanti, 2014).

The cultures were kept in a Bio Chemical Oxygen (BOD) incubator where cultures were allowed to grow at 3K light and 18-20° C under 50 µMol photons $m^{-2}sec^{-1}$ on a 14:10 hr L : D (Complete light : Dark) cycle (Fluorescent light, FL40S : D National) and were growing in an exponential phase for 20-22 days. Pure cultures of diatoms were preserved and maintained on DM liquid medium and transferred to fresh medium at a regular interval of 1 month (Gurung *et al*., 2012; 2013).

Preparation of diatom frustule for microscopic study

The diatom cells were cleaned by acid to remove the organic matrix present external to the cell wall (Hasle and Fryxell, 1970). The cleaned frustule valves were then stored in ethanol to avoid contamination and bacterial growth. The structural morphology of the cleaned diatom frustules were examined by Scanning Electron Microscope JEOL JSM – 6360. The cleaned frustules were partly mounted on brass stubs and coated

Fig.1. Map showing the sampling sites (Source: www.mapsofindia.com).

with gold for SEM analysis and digital images were taken using the system.

RESULTS AND DISCUSSION

SEM analysis

The ultra-structure and morphology of nano-porous silica frustules of the freshwater diatoms were investigated from the silica rich sites- *Chapanala* and *Jiajuri* of Nagaon district of Assam. The structural morphology of the acid treated cleaned frustules were examined by SEM and the images along with their nanopore sizes are described.

Class: Bacillariophyceae

Order: Naviculales

Family: Pinnulariaceae

Genus: *Pinnularia*

Fig. 2. showed that valves are linear to linear-lanceolate with obtusely rounded, subrostrate apices. Striae chambered and with abrupt transition. The external proximal raphe ends dilated, bent slightly. Length of the valve ranges from 30-48µm and width ranges from 5.5-7.5µm. From the SEM images, the diatom was identified as *Pinnularia* sp. having the silicon pore sizes of ~81nm.

Order: Bacillariales

Family: Naviculaceae

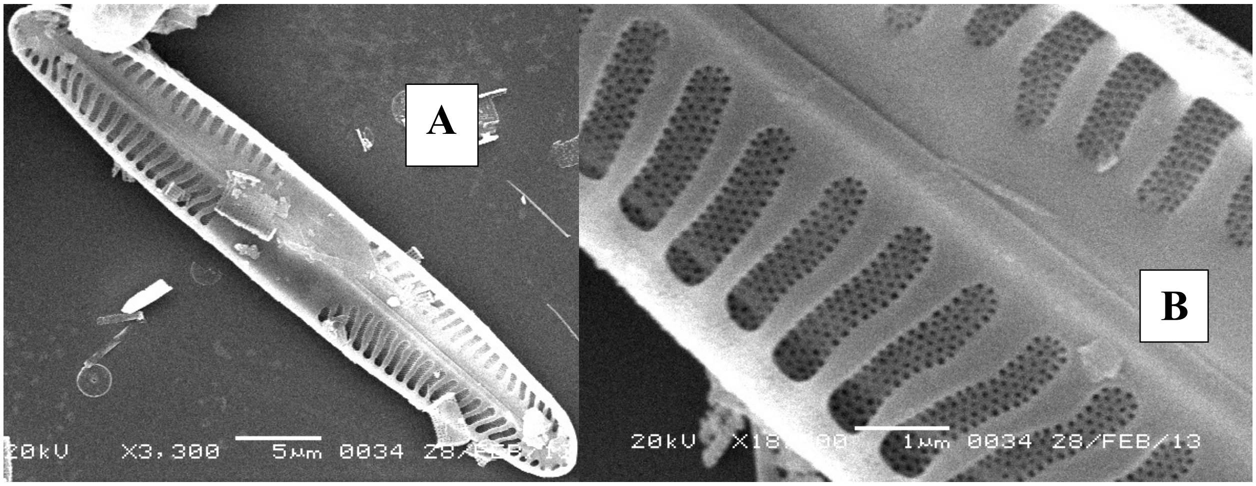

Figure 2. SEM micrographs of *Pinnulariainterrupta*(A) Full view (B) detail surface of the valve showing

Genus: *Navicula*

Fig. 3. showed a scanning electron micrograph (SEM) where, it was observed that the frustules of the diatom was rhombic-lanceolate with cuneate apices. Length of the valve ranges from 75.5-90μm and width ranges from 17-20μm. From the SEM images, the diatom was identified to be *Navicula* sp. The silica nanopores of this diatom species showed ~63nm in size.

Order: Achnanthales

Family: Achnanthaceae

Genus: *Achnanthidium*

Fig. 4. showed that frustules are monoraphid, valves are linear-lanceolate with slightly capitate ends. Striae usually uniseriate and radiate throughout both valves. Length of the valve ranges from 6-21μm and width ranges from 1.5-3μm. From the SEM images, the diatom was identified to be *Achnanthidium* sp. having silica nanoporous structure of frustule of ~140-160nm.

Order: Bacillariales

Family: Bacillariaceae

Genus: *Nitzschia*

Fig. 5. revealed that the valves are lanceolate with sides parallel and tapering rapidly at the poles, terminating with subcapitate apices. Striae barely visible. Length of the valve ranges from 12-42μm and width ranges from 3.5-4.5μm. From the SEM images, the diatom was identified as *Nitzschia* sp. having the silicon pore sizes of ~60-65 nm.

Order: Bacillariales

Family: Eunotiaceae

Genus: *Eunotia*

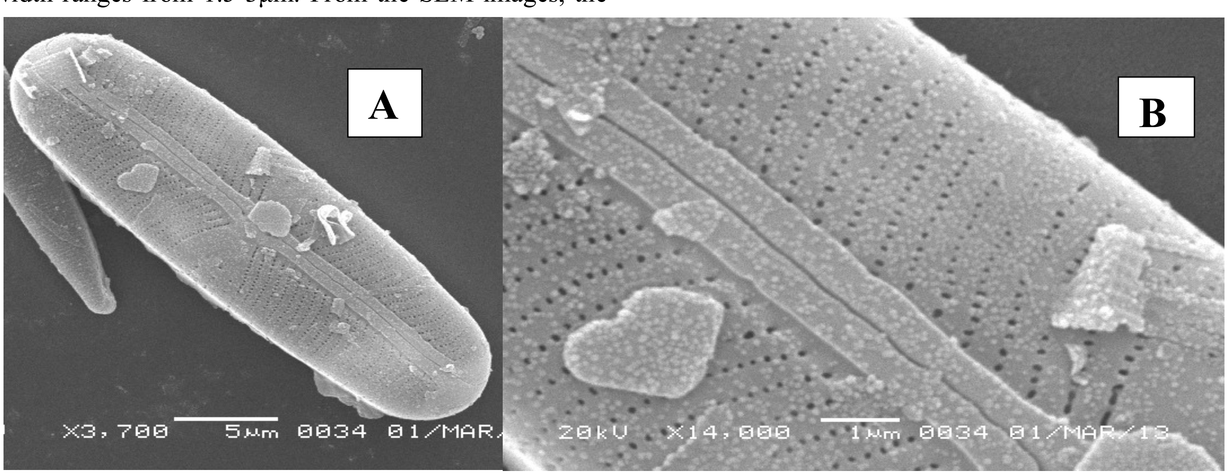

Figure 3. SEM micrographs of *Naviculabacillum* (A) Full view (B) detail surface of the valve showing pores.

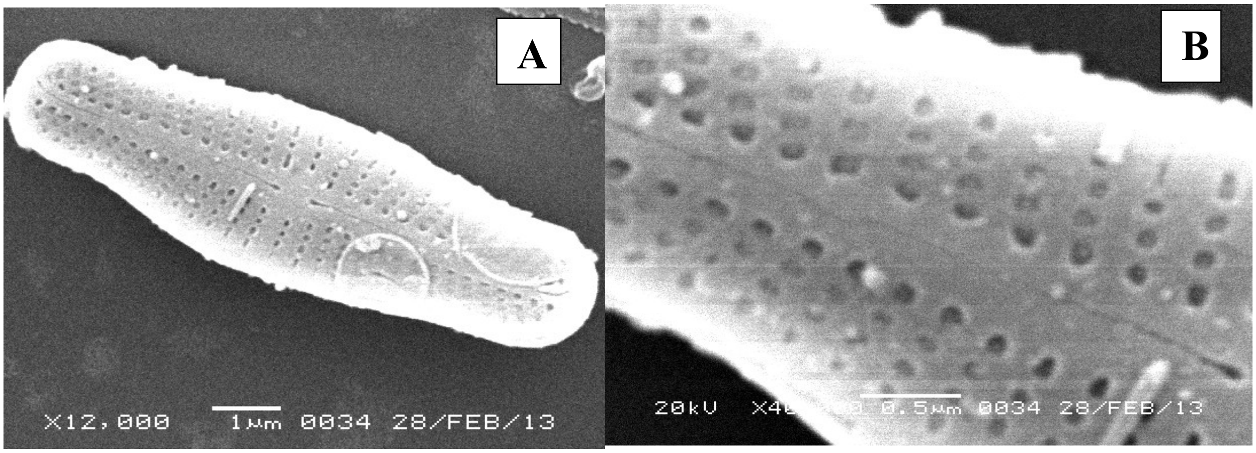

Figure 4. SEM micrographs of *Achnanthidiumminutissumum* (A) Full view (B) detail surface of the valve showing pores.

Fig. 6. revealed that the valves are arched slightly, the dorsal margin convex and narrowing towards the ends and ventral margin concave. Striae radiate at apices. Length of the valve ranges from 21-90µm and width ranges from 5.6-7.2µm. From the SEM images, the diatom was identified to be *Eunotia* sp. which revealed ~150-170 nm of pore sizes.

CONCLUSION

Inspite of immense potentiality of diatoms in nanoengineering and technology, no any proper scientific exploration and exploitation of the freshwater diatoms has been carried out from North-Eastern part of India. Silica rich soil has a distinctive type of ecological habitat supporting specific types of diatoms with different type of features. Diatom frustules display a diversity of patterns and structures at the nano to millimetre scale. In this study, we observed very exciting results in case of *Pinnularia, Navicula* and *Nitzschia* species where their nanoporous silica sizes are less than 100 nm. Nanoporous silica with less ≤ 100 is considered as excellent materials for wide range of applications in IT based industries. Further, as these particles are biologically generated, so they are most stable, cost-effective and eco-friendly. The two other diatoms namely, *Achnanthidium* and *Eunotia* are also showing considerable range of nanoporous silica of ~ 150 nm over their frustules. Their varied geometries and nanopore sizes offer a wide range of attributes for exploitation in nanotechnology based industries. The highly ordered 3D porous silica nanostructures hold a promising vicinity for the biological fabrication of

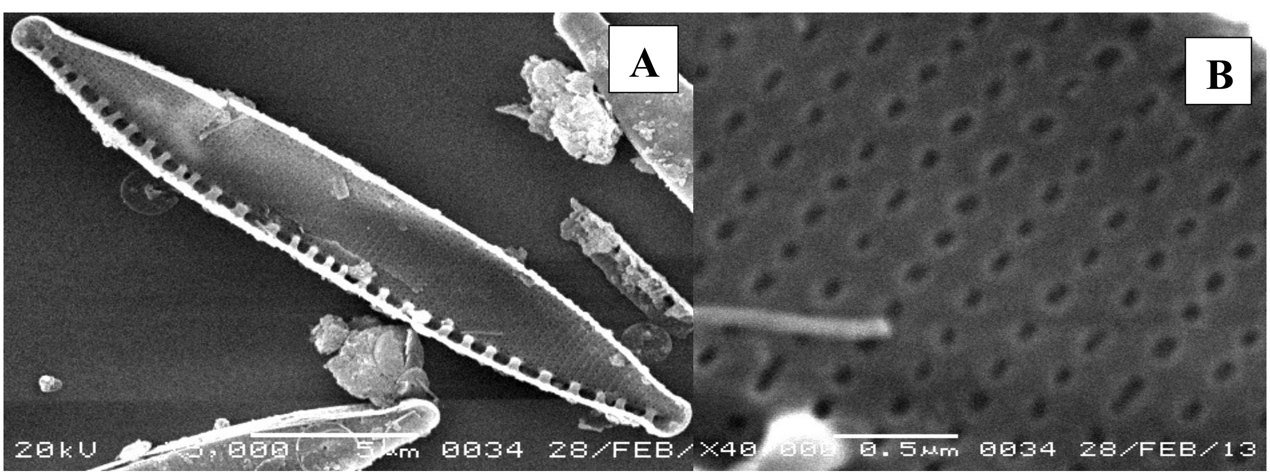

Figure 5. SEM micrographs of *Nitzschiapalea* (A) Full view (B) detail surface of the valve showing pores.

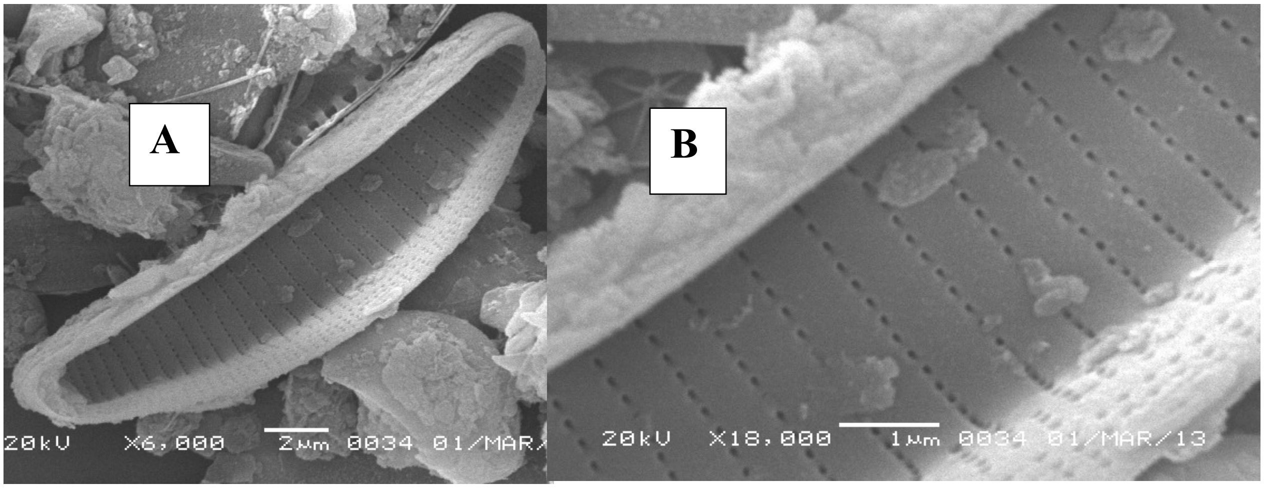

Figure 6. SEM micrographs of *Eunotiasubarcuatioides* (A) Full view (B) detail surface of the valve showing pores.

nanostructured devices and materials from these silica rich sites. For that, more characterization is needed for confirmation and authentication.

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REFERENCES

Beakes GW, Canter HM and Jaworski GHM. 1988. Zoospore ultrastructure of *Zygorhizidium affluens* and *Z. planktonicum*, two chytrids parasitizing the diatom *Asterionella formosa.* Canadian J Bot.,66(6): 1054-1067.

Borgohain D and Tanti B. 2014. Diversity of freshwater diatoms from few silica rich habitats of Assam, India. J. Res. Bio., 4(1): 1162-1173.

Borgohain D and Tanti B. 2014. Seasonal variations of freshwater diatoms in the silica rich soils of Assam. J. Res. Plant Sci., 3(1): 242-248.

Borpuzari P. 2012. Ministry to exploit silica reserves in N-E. The Financial Express, 20 March.

Bozarth A, Maier UG and Zauner S. 2009. Diatoms in biotechnology: modern tools and applications. App Microbiol Biotechnol., 82(2): 195-201.

Gordon R and Parkinson J. 2005. Potential roles for diatomists in nanotechnology. Journal of Nanoscience and Nanotechnology. 5: 35-40.

Gurung L, Tanti B, Buragohain AK and Borah SP. 2012. Studies on the freshwater diatom diversity in Deepar Beel, Assam, India. J Assam Sci Soc., 53(2): 1-6.

Gurung L, Buragohain AK, Borah SP and Tanti B. 2013. Freshwater diatom diversity in Deepor Beel – a Ramsar site. J. Res. Plant Sci., 2(2):182-191.

Hasle GR and Fryxell GA. 1970. Diatoms: cleaning and mounting for light and electron microscopy. Transactions of the Americans Microscopical Society. 89 (4): 469-474.

Montsant A, Aheshwari U, Bowler C. and Lopez PJ. 2005. Diatomics: towards diatom functional genomics. Journal of Nanoscience and Nanotechnology. 5: 5-14.

Round FE, Crawford RM and Mann DG. 1990. The Diatoms: Biology and Morphology of the Genera, Cambridge University Press. p. 747.