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14. ABSTRACT
Diatom cell walls have many potential applications. So far large-scale tests on applications have been conducted using diatomaceous earth. Diatomaceous earth consists of fossilized diatom cell walls, which have non uniform size with a lot of debris and a large variability from batch to batch; these attributes prevent development of a standardized process. To our knowledge this effort was the first attempt to produce kilogram quantities of cell walls of a single species of diatom. Our goal was to produce 1.08kg of clean diatom cell walls for 6 species of diatoms that have different size and shape. We produced 8.5 kg of clean diatom cell walls from 7 different species of

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Report Title

Final Report: Diatom-Based Material Production Demonstration

ABSTRACT

Diatom cell walls have many potential applications. So far large-scale tests on applications have been conducted using diatomaceous earth. Diatomaceous earth consists of fossilized diatom cell walls, which have non uniform size with a lot of debris and a large variability from batch to batch; these attributes prevent development of a standardized process. To our knowledge this effort was the first attempt to produce kilogram quantities of cell walls of a single species of diatom. Our goal was to produce 1.08kg of clean diatom cell walls for 6 species of diatoms that have different size and shape. We produced 8.5 kg of clean diatom cell walls from 7 different species of diatoms. This effort provides proof-of-principle that diatom cell walls can be produced at large scale. The key areas for additional development are further improvements in the process used to clean the cell walls, and cultivation of more species to expand the size and shape of available diatom cell walls.

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Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

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NAME

Total Number:

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Names of other research staff

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David Hazlebeck	0.00
Matthew Haeger	0.02
Sean Hoban	0.05
Leila Kamakele	0.06
Jeff Kataoka	0.08
Randi Keipper	0.16
Brendan Lagather	0.17
Stephen Woods	0.10
Rodney Corpuz	0.13
Aga Pinowska	0.14
Cody Clifton	0.11
Hoang Yen Nguyen	0.44
Kate Walli	0.12
Gabriela LaChance	0.03
FTE Equivalent:	**1.61**
Total Number:	**14**

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

We developed a process for cleaning large quantities of diatom cell walls for use in advanced materials applications.
We developed 4 new growth media recipes for diatoms.
We developed methods for large-scale cultivation and harvesting for 6 different diatoms.

Technology Transfer

Diatom cell walls will be sent to Cathleen Fischer at Dresden University of Technology for testing as a substrate for catalysis.
Material is available for any other testing.

GLOBAL ALGAE INNOVATIONS

March 8, 2016

ARO CAM
Dr. Robert Mantz
Army Research Office
3200 Chapel Hill Nelson Blvd, Suite 206
Durham, NC 27709

CC: DARPA Program Manager
Dr. David McQuade

Ref: W911NF-15-2-0012

Subject: Final Technical Report

Dear Dr. McQuade and Dr. Mantz:

Global Algae Innovations is pleased to provide the enclosed final technical report.

Should you have any questions please contact me at 808-212-4509, facsimile 877-361-0310, or email: agapinowska@globalgae.com.

Sincerely,

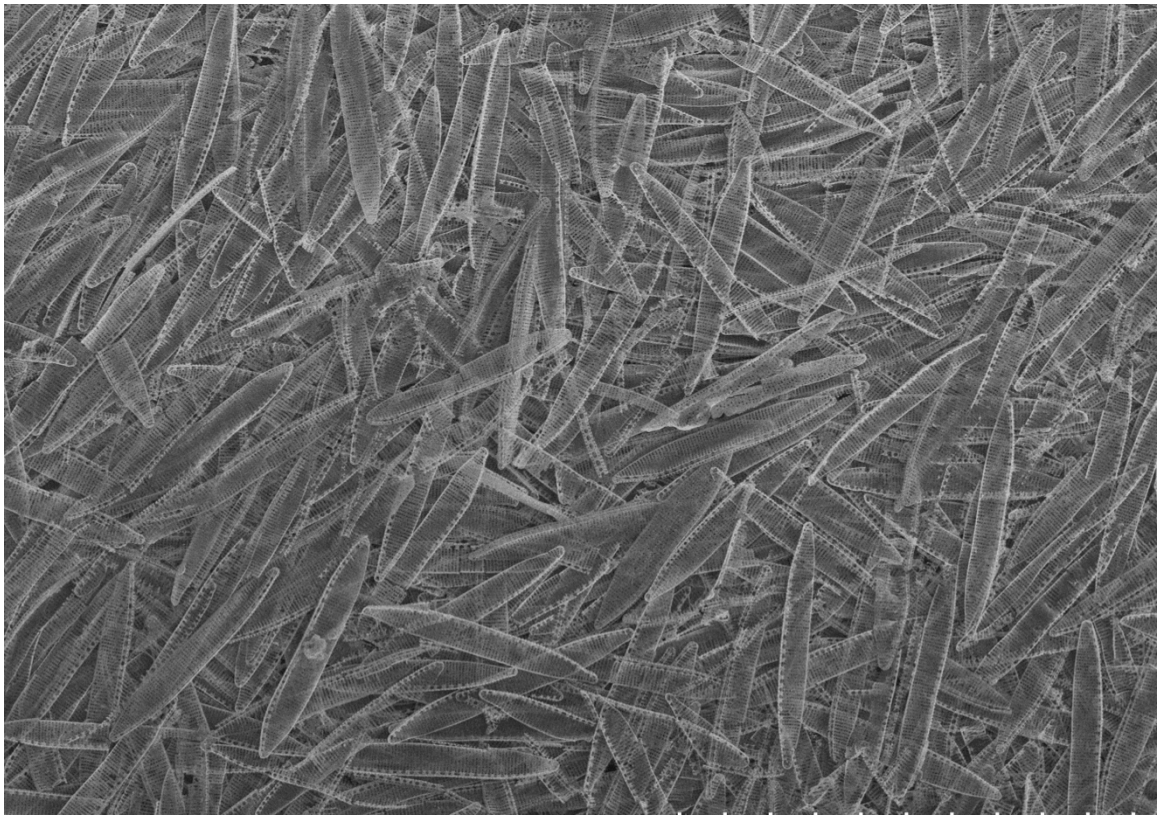
GLOBAL ALGAE INNOVATIONS

Submitted By: Dr. Agnieszka Pinowska
Lead Phycologist

Final Technical Reports

1403-F-20160308-01

March 2016



GLOBAL ALGAE INNOVATIONS

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Diatom-Based Material Production Demonstration
Final Report
Global Algae Innovations, Inc.
March 8, 2016

Foreword

Diatoms are naturally occurring nanoparticles with very divers shapes and size (5-160µm). The interest in use of diatom cell walls in many applications has exploded in the last 3 years with a large increase in the number of papers published using diatom cell walls for various nano applications. They are potential building materials for gas sensors, new optical devices, optical communication networks, passive wave guides, diffraction elements for solar cells, photonic crystal lasers, LED lights, high performance supracapacitors, nano porous materials, nano photo electrodes and nano particle assembly centers. They can also be used as drug delivery capsules, nano medical devices and insecticides. The interest in use of diatom cell walls had just begun and majority of the tests were done on small amounts of material cultivated in the lab or using diatomaceous earth. Analysis of number of papers published on diatom nanotechnology indicates steady increase with new fields like optical applications added in the last few years (Fig. 1).

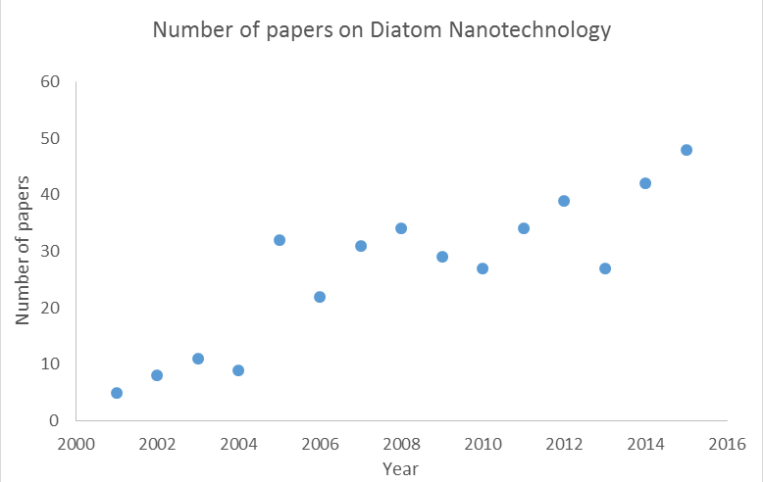

Fig. 1 Number of papers published on using diatoms for nanotechnology since year 2000.

The vast majority of these researchers have little or no experience in diatom cultivation or cell wall purification. Ready availability of a wider range of diatom cell walls is needed to facilitate development of applications that utilize these unique materials. The ability to produce and clean significant quantities of diatom cell walls, including multiple cell wall geometries, was demonstrated in this project. The project is ahead of its time in that the applications for these unique materials are still being developed, so there is no immediate commercial market for the materials; there is a little bit of a 'chicken and egg' phenomena in that limited availability of diatom cell walls constrains the research on

applications, and the need for research on applications has constrained the development of a commercial market.

A program is needed to produce and distribute a wide array of diatom cell wall geometries to spur technology advances in defense and commercial fields based on application of these unique materials. Such a project would not only advance current research on diatom cell wall applications, but could be expected to expand the number of applications as more material scientists and engineers become aware of the unique properties, the wide range of potential geometries, and the ready availability of materials for testing.

Diatom cell walls are a natural product that is fully biodegradable, renewable and environmentally friendly. Diatom cell walls are not toxic to people or animals, and the life cycle of these materials is already well studied. This is in a large contrast to many synthetic nanoparticles, which represent a potential health and environmental hazard. The life cycle of synthetic nano particles is unknown, but harmful effects to people and the environment has already been observed. If environmental concerns limit the use of some synthetic nanoparticles, then the ready availability of diatom cell wall material would offer researchers an environmentally friendly way to achieve the same technology advance.

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Statement of the problem studied and summary of results

Diatom cell walls have many potential applications. So far large-scale tests on applications have been conducted using diatomaceous earth. Diatomaceous earth consists of fossilized diatom cell walls, which have non uniform size with a lot of debris and a large variability from batch to batch; these attributes prevent development of a standardized process. All testing on use of diatom cell walls from cultured diatoms has been conducted on small samples produced in the lab or in small bioreactors and only gram quantities of cell walls have previously been produced.

To our knowledge this effort was the first attempt to produce kilogram quantities of cell walls of a single species of diatom. Our goal was to produce 1.08kg of clean diatom cell walls for 6 species of diatoms that have different size and shape. We produced 8.5 kg of clean diatom cell walls from 7 different species of diatoms. This effort provides proof-of-principle that diatom cell walls can be produced at large scale. The key areas for additional development are further improvements in the process used to clean the cell walls, and cultivation of more species to expand the size and shape of available diatom cell walls.

Brief introduction to diatom cell wall

Diatom cell wall (also called frustule) is made of amorphous silica and chitin and associated with it proteins and other polysaccharides. From the outside it is covered by proteins (Fig. 2). Frustule looks like a box and it consists of two main parts epitheca and hypotheca. Epitheca and hypotheca consist of a valve and cingulum. Cingulum is made of multiple girdle bands (Fig. 3).

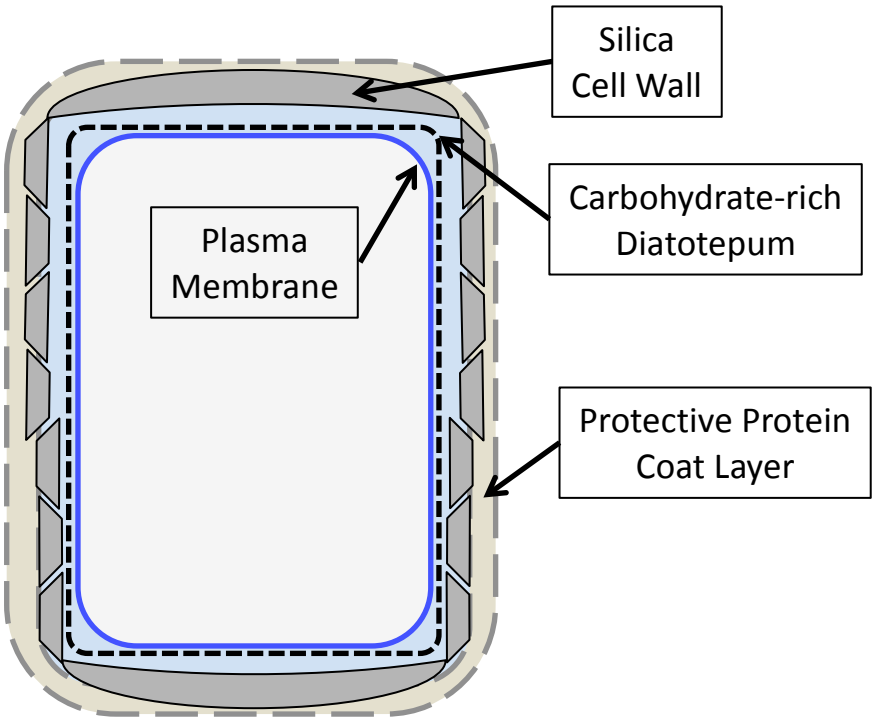


Fig. 2 Diagram showing diatom cell membrane, diatotepum (chitin and other carbohydrates), silica cell wall and protein coat layer (M. Hildebrand).

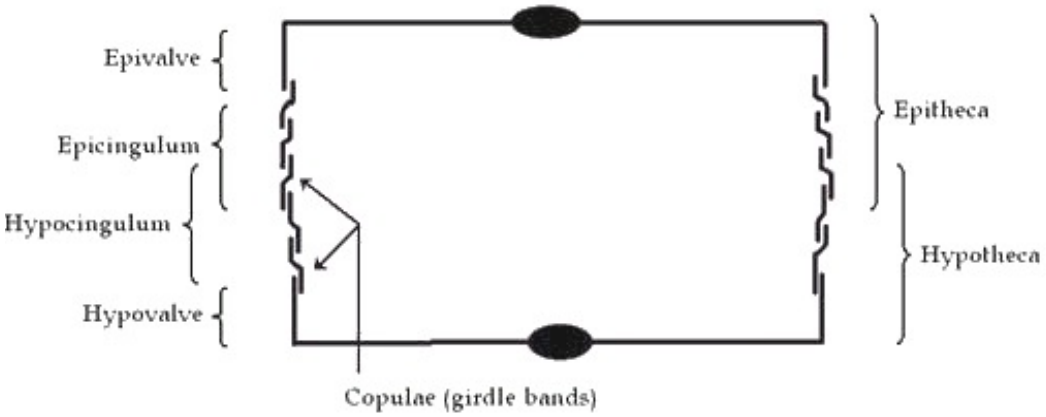
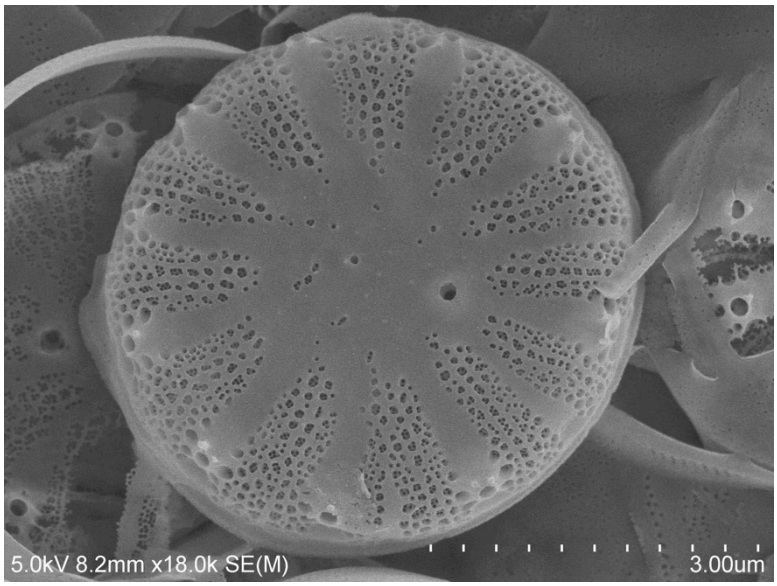


Fig. 3 Schematic of diatom cell wall.

After organic material is removed diatom cell wall will usually separate into epivalve, hypovalve and girdle bands. Diatom epivalve and hypovalve are the most silicified and have the most intricate structures. Diatom valves are used to identify diatoms based on their morphology. Diatom valves have very intricate 3-dimetnioal poroid system on their surface that contributes to the large surface of diatom cell wall (Fig. 4). Girdle bands also have poroids but their pattern is less intricate. Pores are smaller than poroids and they are all covered with a thin layer of silica. This layer is often removed during cleaning of diatoms.

a.



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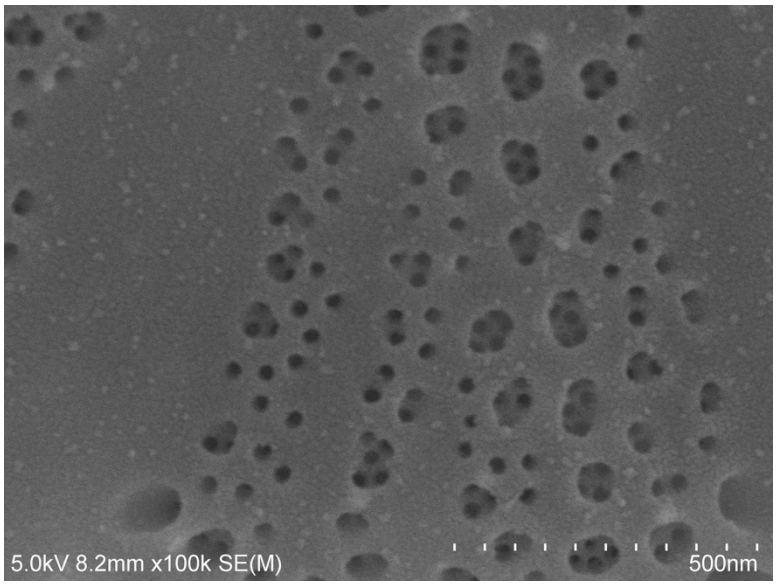


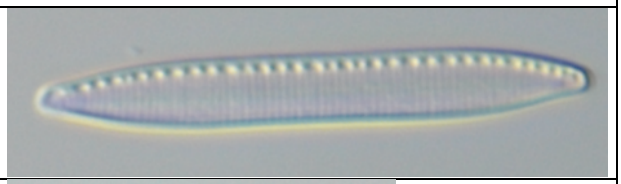
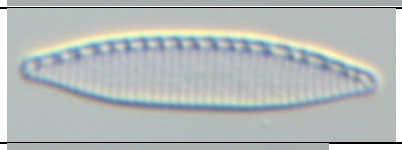
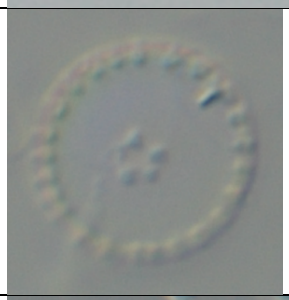
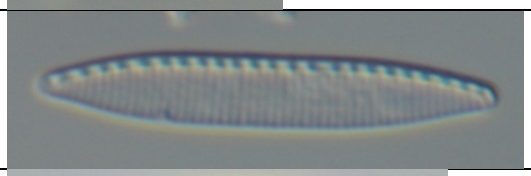
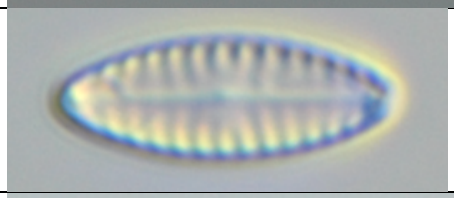
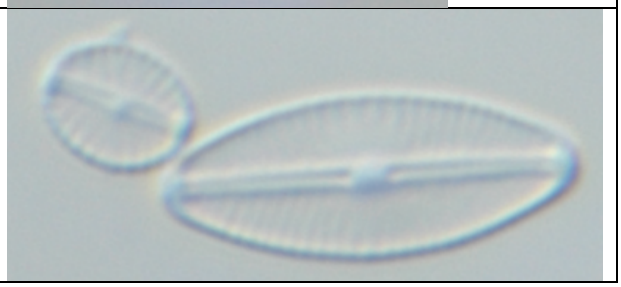
Fig. 4 GAI-209 valve (a) and close up of valve poroids (b).

Summary of the most important results

Strains

We selected 7 strains that were successfully cultivated in high density cultures. We selected 5 pennate (long) diatoms and 2 centric diatoms. Strains differed in length from 4 to 30 µm. The light microscopy pictures of the clean cell walls are shown in Table 1. For these microscopy analyses, the diatoms were boiled in nitric acid, rinsed with deionized water and mounted in Naphrax resin.

Table 1 Diatoms selected for scale up showing acid cleaned cell walls.

Strain	Cell size	Media	Picture of acid cleaned diatom valves
GAI-216 *Nitzschia* sp. pennate	27.1-30.2µm long and 3.3-4.9µm wide	Bicarbonate	
GAI-205 *Nitzschia* sp. pennate	18-20µm long and 3.2-4µm wide	Bicarbonate	
GAI-209 *Cyclotella* sp. centric	7.8-9.0µm in diameter and 7.2-9.2µm long	Freshwater	
GAI-005 *Thalassiosira weissflogii* centric	6.0-9.8µm in diameter and 10-16.2µm long	Seawater	
GAI-229 *Nitzschia* sp. pennate	13.1-22.4µm long and 2.8-5.6 µm wide	Bicarbonate	
GAI-208 *Navicula* sp. pennate	8-12.5µm long and 4-5µm wide	Seawater	
GAI-215 *Navicula* sp. pennate	4-12.4µm long and 3.5-5.1µm	Freshwater	

In order to obtain high growth rates and high-density cultures we developed new media recipes for the selected strains. Diatoms were cultivated in 96-well microplates and their growth was monitored using absorbance at 750nm (Fig. 5).

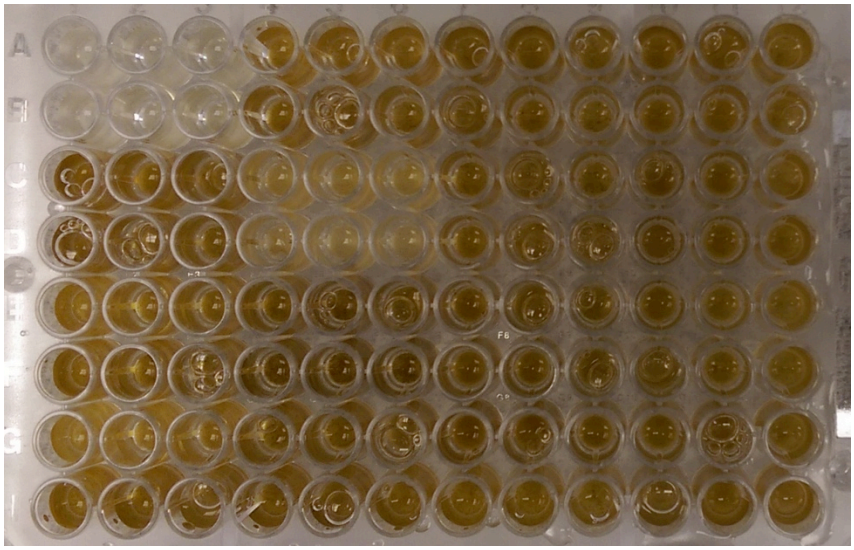


Fig. 5 96-well microplate experiment for media optimization. All treatments were run in triplicate high growth treatments are dark brown with a lot of bubbles showing intense photosynthesis.

Grow out and harvest

Diatom culture scale-up was started in flasks, then they were cultivated in the laboratory in carboys and finally at the farm in open ponds (Fig. 6). All strains from Table 1 but GAI-215 were cultivated at the farm in open ponds. The method to cultivated GAI-215 was developed to late in the project to take it out into the open ponds at the farm. GAI-215 was successfully cultivated in the carboys at the laboratory. The total amounts of diatoms cultivated are listed in Table 2 For detail table of diatoms cultivated at the farm and at the lab refer to the Appendix.

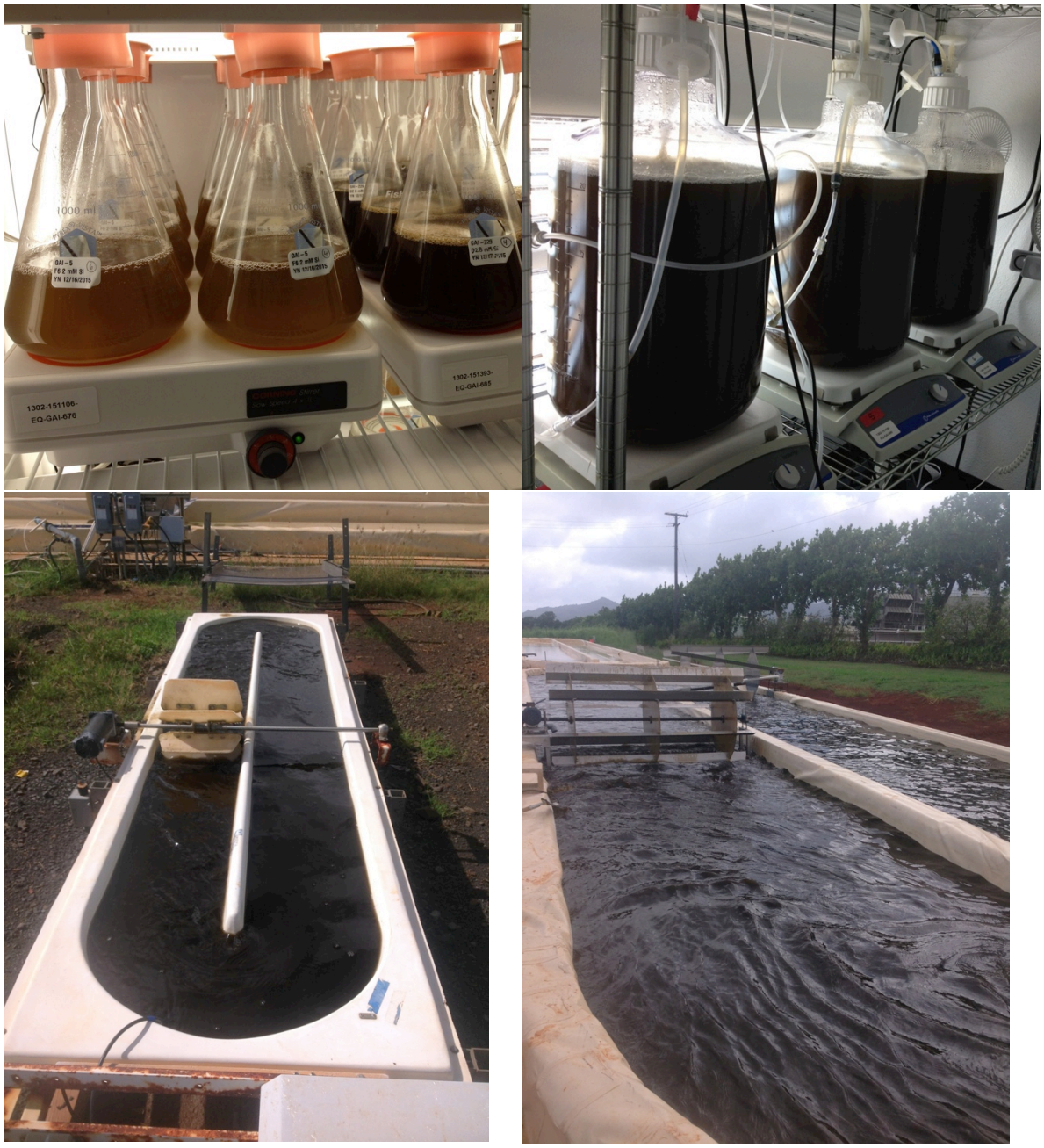


Fig. 6 Cultivation of diatoms in the flasks and carboys at the lab and in open ponds at the farm.

Table 2 Quantitates of algae cultivated.

Species	Minimum deliverables - diatom cell walls	Target mass of clean cell walls	Harvested culture volume (L)	Total DW harvested (g)	Total AFDW harvested (g)
GAI-216 *Nitzschia* sp.	1000g	1000g	47,500	31,700	23,750
GAI-205 *Nitzschia* sp.	20g	300-400g	8,500	5,100	3,569
GAI-209 *Cyclotella* sp.	10g	10g	552	242	154
GAI-005 *Thalassiosira weissflogii*	10g	10g	793	725	297
GAI-229 *Nitzschia* sp.	20g	20-100g	621	660	372
GAI-208 *Navicula* sp.	20g	20-100g	500	415	250
GAI-215 *Navicula* sp.	0g	0.5g	40	15	10

Small samples (20-60L) were harvested at the laboratory using WVO Extreme Raw Power Biodiesel Centrifuge and large samples (over 100L) were first concentrated using Zobi harvest systems at the farm and then the concentrate was centrifuged using Evodos 20 centrifuge at the KAF or using the WVO Centrifuge at the lab (Fig. 7). There were 2 membrane systems used. GAI-216 and GAI-205 were processed through GAI's membrane Pilot system. The remaining batches were processed through GAI's Mini system. Final paste was approximately 20% solids (Table 3). The harvested paste was frozen or refrigerated and stored until it was for processed into clean cell walls.

Fig. 7 Harvesting of diatoms using WVO Centrifuge and Mini membrane system.

Table 3 Dewatering and centrifuging steps for processing of diatom biomass.

Species	Dewatering system	Post membrane concentration % solids	Type centrifuge	Paste concentration % solids
GAI-216	Pilot, 20m^3/h	1.8	Evodos 20	19.7
GAI-205	Pilot, 20m^3/h	1.1	Evodos 20	21.2
GAI-209	Mini, 20L/h	2	WVO Centrifuge	18.1
GAI-005	Mini, 20L/h	2	WVO Centrifuge	20.2
GAI-229	Mini, 20L/h	2	WVO Centrifuge	19.5
GAI-208	Mini, 20L/h	2	WVO Centrifuge	20.3

Cleaning

Frozen diatom paste was defrosted and diatom cell walls were cleaned. A cell wall cleaning method was developed by modifying the process described in Jantschke (2014). This modified method was used at our laboratory and at the farm. Samples cultivated at the lab were cleaned at the lab and samples cultivated at the farm were cleaned at the farm. We also took a small sample of farm cultivated GAI-208 and cleaned it at the lab.

Laboratory method

First step was to soak the diatom biomass in a SDS/EDTA buffer (69mM SDS, 100mM EDTA, 307mM NaOH, pH 8) while mixing on an orbital shaker. Next this solution was transferred to a rotary evaporator mixer and heated to 95°C for 1 hour. From 1 to 3 SDS/EDTA cleaning steps were performed. Cell walls were washed 3 or 4 times with deionized water between the steps. Next cell walls were cleaned with 35% peroxide in the rotary evaporator mixer heated to 95°C for 1 hour (Fig. 8 and Fig. 9). From 1 to 3 peroxide cleaning steps were used depending on species and cultivation conditions. Cell walls were washed 4 or 5 times with deionized water between the steps. If the peroxide step did not show any foaming, then the material was considered cleaned. The final material was rinsed at least 3 times with deionized water.

Fig. 8 Rotary evaporator used for cleaning of diatom cell walls at the laboratory.

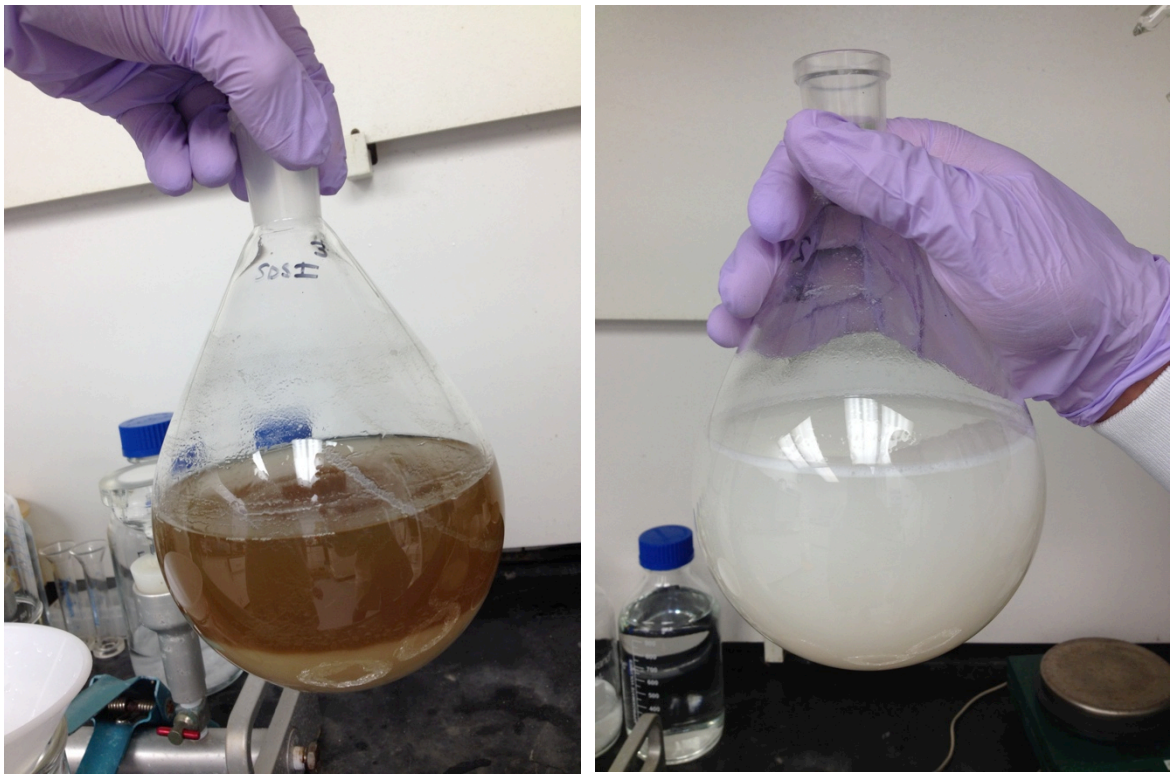


Fig. 9 Cleaning of diatom cell walls at the laboratory with SDS/EDT (left) and peroxide (right).

During cleaning process material was examined under the light and fluorescent microscope. Subsequent EDTA/SDS rinses removed more and more of the organic material. Hydrogen peroxide treatment removed majority of organic material and under light microscopy material was clear (Fig. 10).

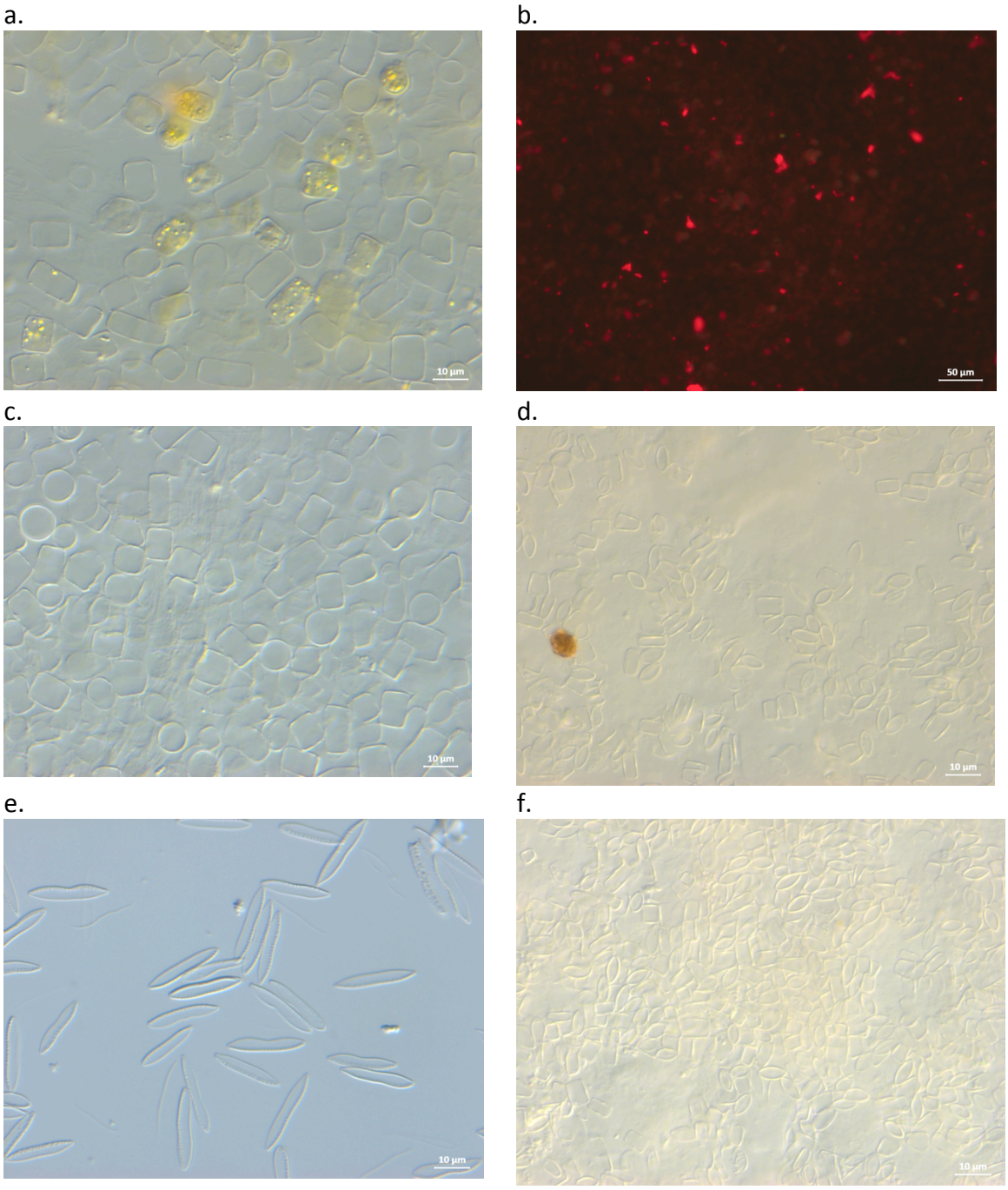


Fig. 10 Light and fluorescence microscopy images of diatom cell walls after EDTA/SDS and peroxide cleaning steps. GAI-005 after single EDTA/SDS cleaning (a and b showing chlorophyll fluorescence). GAI-005 after 3 EDTA/SDS cleaning steps (c). GAI-208 after three ETDA/SDS and one peroxide cleaning steps (d). GAI-216 after three ETDA/SDS and one peroxide cleaning steps (e). GAI-208

after three ETDA/SDS and three peroxide cleaning steps. GAI-005 was cultivated at the lab and GAI-208 and GAI-216 were cultivated at the farm. Panel d shows a granule of Kauai red soil.

We also analyzed ash content of the final material and it varied from 91 to 97% depending on species. We tried to calcinate the material to remove the remaining organic material or possibly other residue leftover from cleaning process but our calcinated material was clumping and it was difficult to re-suspend it back in water. We decided to keep our final clean product in deionized water and skip the calcination step.

Farm method

Diatoms were processed in a 200L or 18L stainless steel tank (Fig. 11).
Each diatom batch was cooked in a compatible tank reactor with EDTA/SDS solution for 30 min at temp, 95°C, then cooled to ambient. This process was called the P1 stage. Once temperature was below 24°C the supernatant was decanted. This step was performed a total of 3 times. The washed biomass was then rinsed with farm water a total of 3 times. For each successive rinse, a mixer was used after each decant step to re-suspend the bio-silica from gravity settling. The next step in the process uses 35% Hydrogen peroxide to remove any residual organics. We called this the P2 stage. Prior to the peroxide step, each washed and rinsed bio-silica batch was then rinsed again with deionized water one time to remove any trace metals found in the farm ZW water. The P2 step also required a heat soak step to 95°C for 1 hour followed by a cooling stage. Once temperature was below 24°C the supernatant was decanted and peroxide treatment was repeated 2 more times. One main difference during this step required the peroxide to reach temperature first. After target temperature was reached, the bio-silica paste was added to the system. ORP measurements were taken to ensure efficiency of the P2 step. Once the OPR measured below 200, oxidation potential was exhausted. This step was repeated a total of 2 times to ensure all organics available were oxidized. The system was then decanted and rinsed with deionized water. After the rinse was performed 3 times, the contents were removed from the tanks and stowed in 20L tote bins for further cleaning. Table 4 shows more detail of the P1 and P2 stages. GAI-209 material was lost during processing at the farm as it boiled over and spilled.

Fig. 11 Tanks for cleaning of diatom cell walls.

Table 4 P1 and P2 step details for cleaning of diatom cell walls at the farm.

Species	P1 EDTA/SDS:AFDW paste ratio (v/v)	P2 35%H2O2:biosilica ration (v/v)	Reaction volume (L)
GAI-216	4:1	2:1	200
GAI-205	4:1	3:1	200
GAI-209	5:1	4:1	18
GAI-005	5:1	4:1	18
GAI-229	5:1	4:1	18
GAI-208	5:1	4:1	18

Cleaned material was further sieved and settled to remove soil particles and other debris. The four stainless steel sieve sizes that we used were 230 (63 micron), 270 (53 micron), 325 (45 micron) and 400 (38 micron). Each batch was re-suspended to a pumpable concentration, 2% solids. The batch was then fed via aquarium pump to the sieves. A 1.5HP sieve shaker system then facilitated the flow to a clean reservoir. Each batch was then gravity settled and decanted (Fig. 12).

Fig. 12 Setup for sieving diatom cell walls on the left, metal sieve and final sieved cell walls on the right.

Final quantities of diatom cell walls produced

The final amounts of cell walls produced are listed in Table 5. Detailed breakdown of cell walls produced at the farm and at the lab can be found in the Appendix.

Table 5 Quantities of diatom cell walls produced.

Species	Minimum contract deliverables of diatom cell walls (g)	Quantity of cleaned diatom cell walls produced (g)
Nitzschia sp. GAI-216	1000	7181.9
Nitzschia sp. GAI-205	20	1048.1
Nitzschia sp. GAI-229	20	127.8
Navicula sp. GAI-208	20	82.6
Thalassiosira weissflogii GAI-005	10	57.4
Cyclotella sp. GAI-209 and *Navicula* sp. GAI-215 combined	10	10.0
Total quantity of cell walls on ash basis (g)	**1080**	**8507.7**

Material analysis

Final clean material was analyzed using Scanning Electron Microscopy (SEM), Light Microscopy (LM) and Flow Cytometry (FC). We analyzed samples that were cultivated at the lab and at the farm.

For SEM samples were diluted in deionized water and dried on 13mm diameter round Corning Gold Seal cover glass. Samples were then mounted on aluminum stubs with double-stick carbon tape and coated with gold/palladium in a Hummer 6.2 sputter coater. Some samples were coated twice first straight up and second in a 45-degree angle to reduce charging. Some samples were still charging even after double coating indicating some residue from processing of the material. Specimens were viewed and digital images were acquired with a Hitachi S-4800 Field Emission Scanning Electron Microscope at an accelerating voltage of 5.0 kV. We used SEM at Pacific Biosciences Research Center, Biological Electron Microscope Facility at the University of Hawaii at Manoa.

For LM samples were diluted in deionized water and dried on 22x22 mm square Corning 1.5 cover glass. Dried samples were mounted in Naphrax resin and examined under Zeiss Imager A2 research microscope under 40x and 100x objectives giving total magnification of 640x and 1600x. Digital images were taken using Zeiss AxiomCam RHc.

For flowcytometry samples were diluted in deionized water and analyzed on BioRad S3 Cell Sorter using forward (FSC) and side scatter (SSC). The areas of interest were delineated as cell walls and other debris and both populations were sorted into tubes. Sorted samples were settled and analyzed under light microscope.

GAI-216 Nitzschia sp.

The cell wall of GAI-216 was the main diatom cell wall produced. SEM images of material produced on the farm and at the lab show diatom valves and girdle bands (Fig. 13 and Fig. 14). Laboratory material shows open areas where poroids are locate indicating that cleaning process of this sample dissolved the thinnest parts of the cell wall (Fig. 13c). Farm produced material was collected from the top of the processing tank and shows primarily girdle bands (Fig. 14c). Indicating that valves were heavier and settled on the bottom of the tank this suggests that material can be separated into valves and girdle bands by settling. This batch of farm processed material did not show dissolution of the poroids (Fig. 14d).

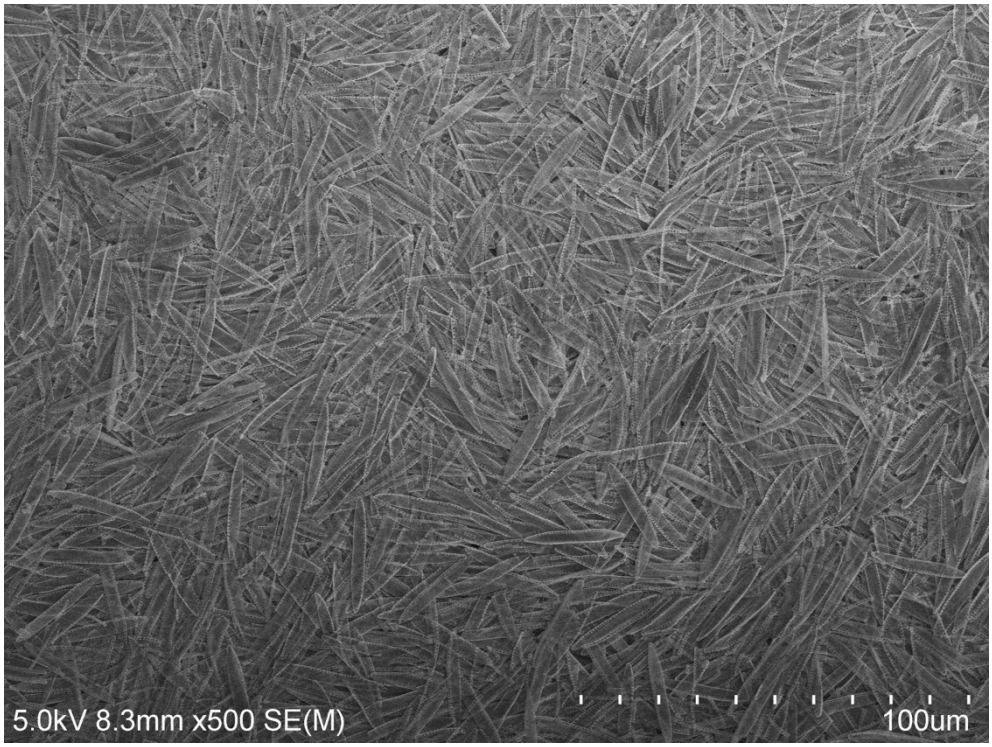
Light microscopy analysis was conducted on the farm material that came from a mixed sample. It shows an even mix of girdle bands and valves (Fig. 15).

Mixed farm sample was also analyzed on a flow cytometer. Sample showed 2 populations: one of large particles and one of small. We sorted out the two populations (R1 and R2) and we examined them under the light microscope. The large population (R1) consisted of valves and some girdle bands. The small population (R2) was of very small particles that were not visible under the light microscope (Fig. 16).

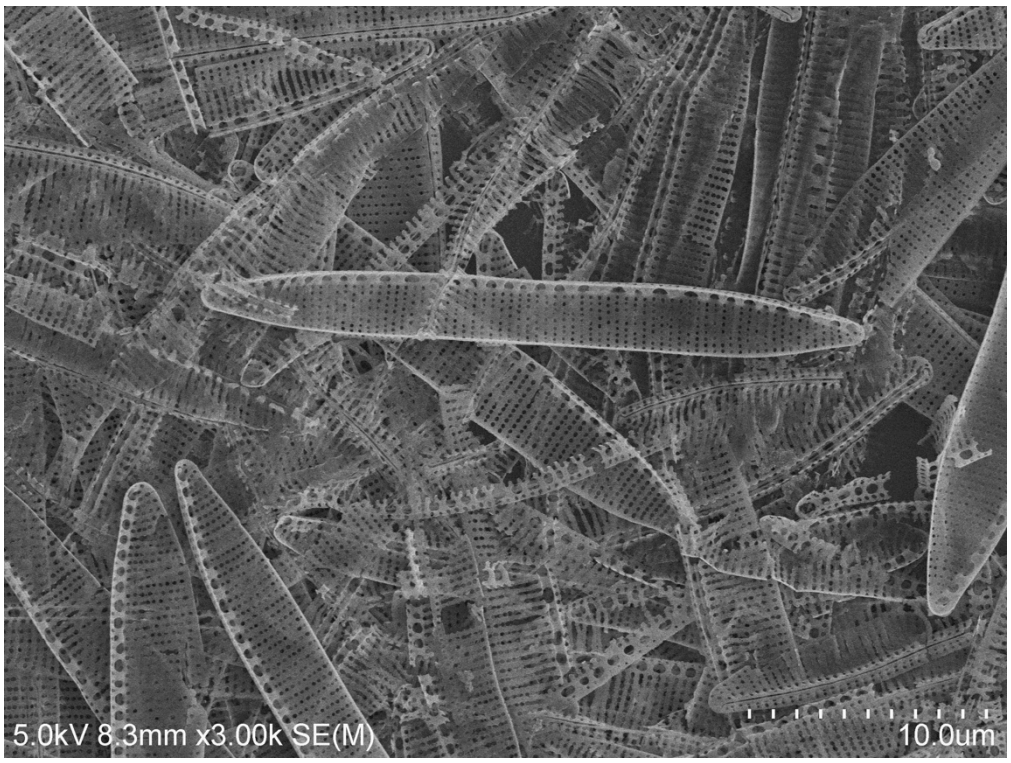
Over all diatoms that were cultivated in freshwater media or media with bicarbonate had very little precipitate and were very clean. Samples had varying amount of girdle bands,

this could be species specific or it is possible that some of the girdle bands were lighter and were washed out during the rinsing process. Farm material was contaminated with red soil particles, but most of it was removed by washing, sieving and settling, the material with final cell walls showing reduced contamination.

a.



b.



c.

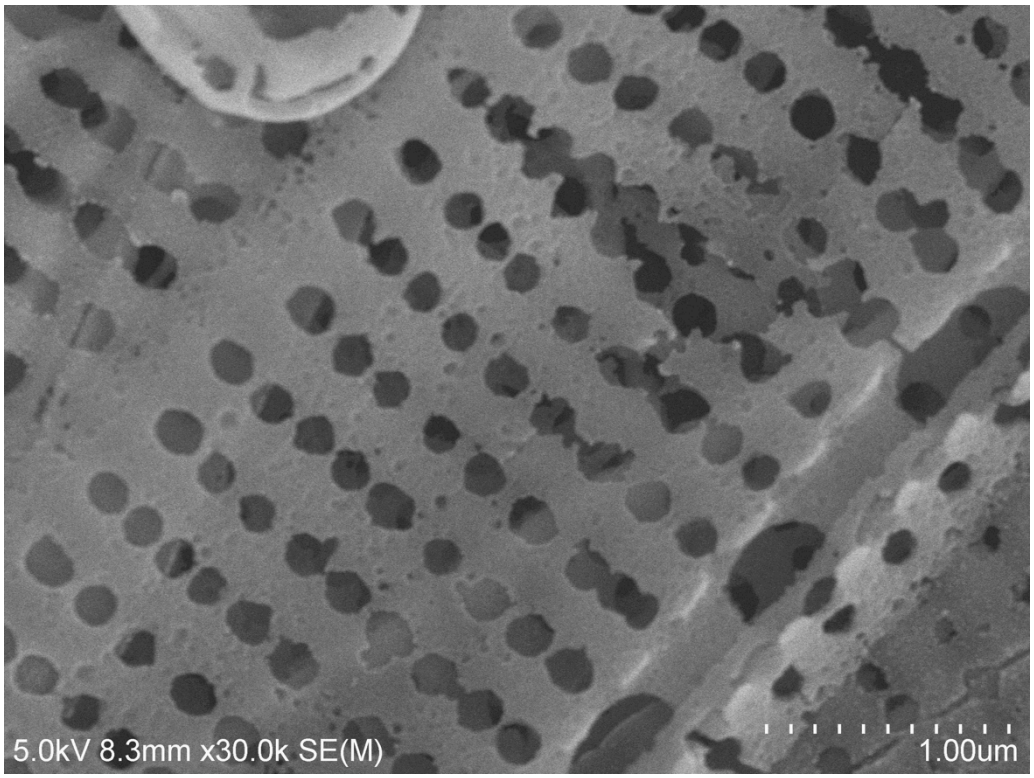
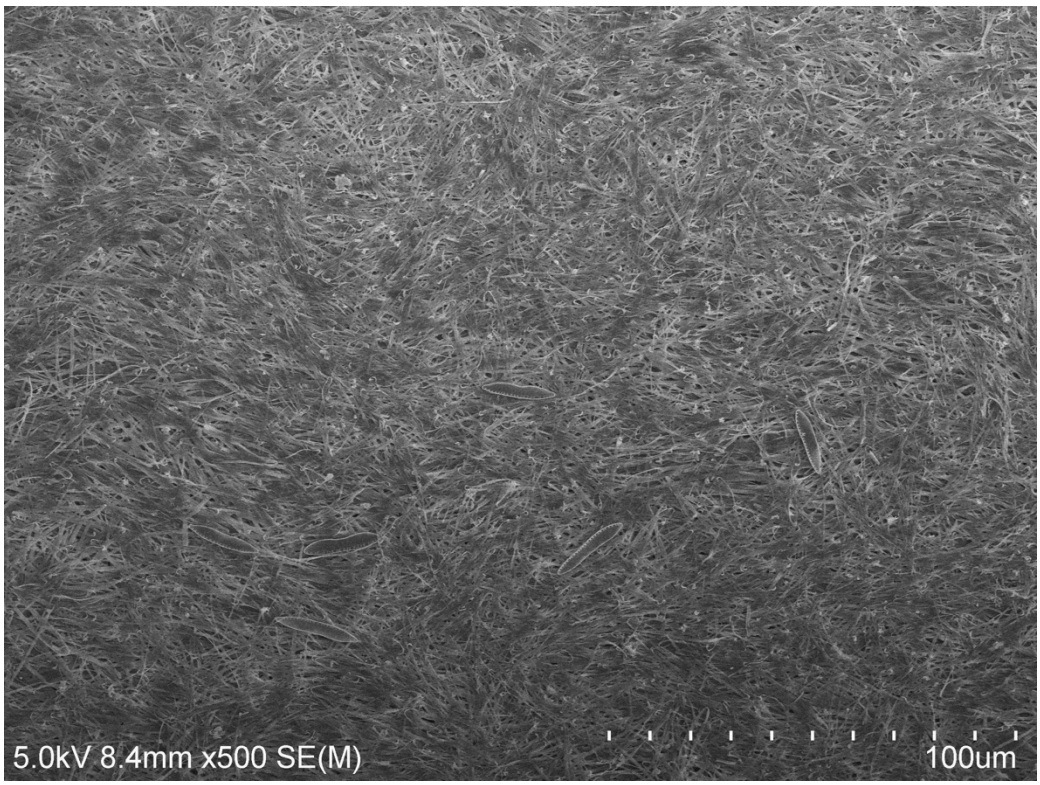
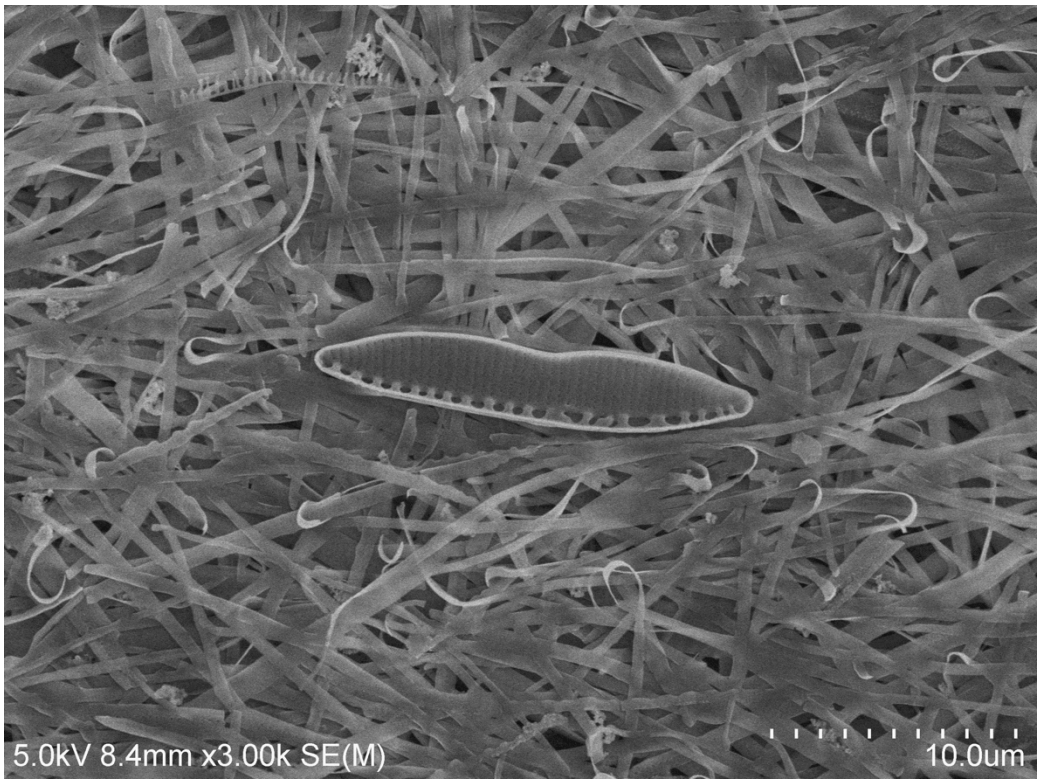


Fig. 13 GAI-216 lab sample showing valves (a,b) and fully open poroids (c).

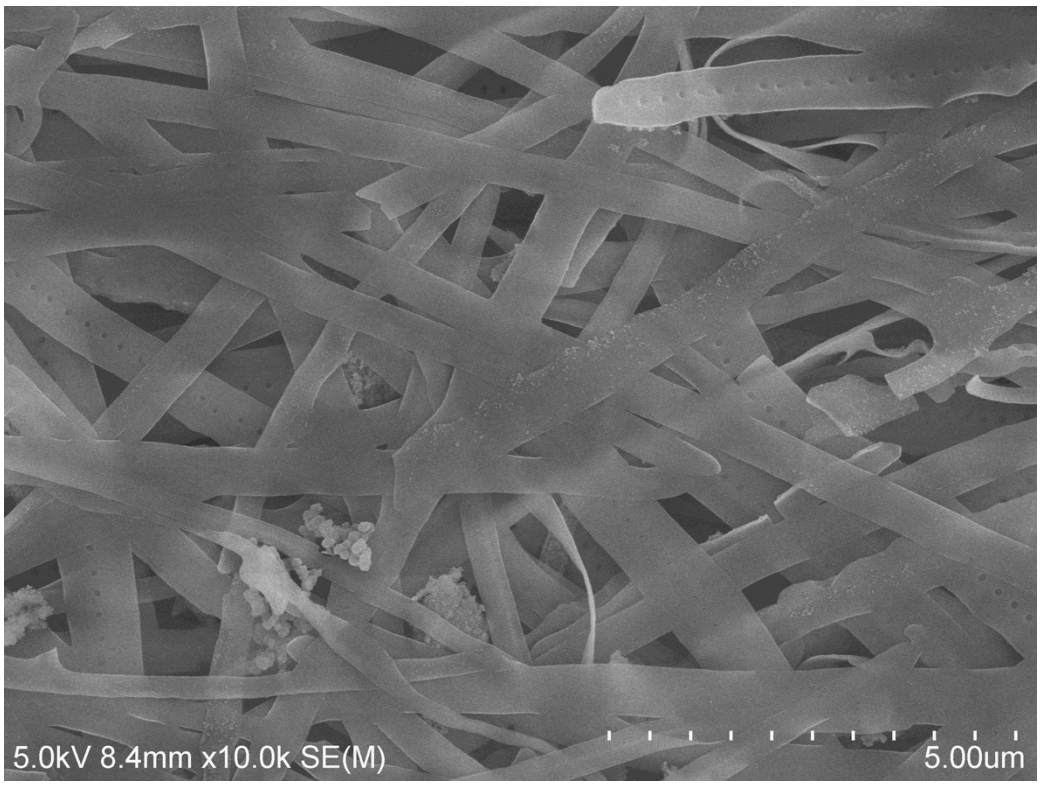
a.



b.



c.



d.

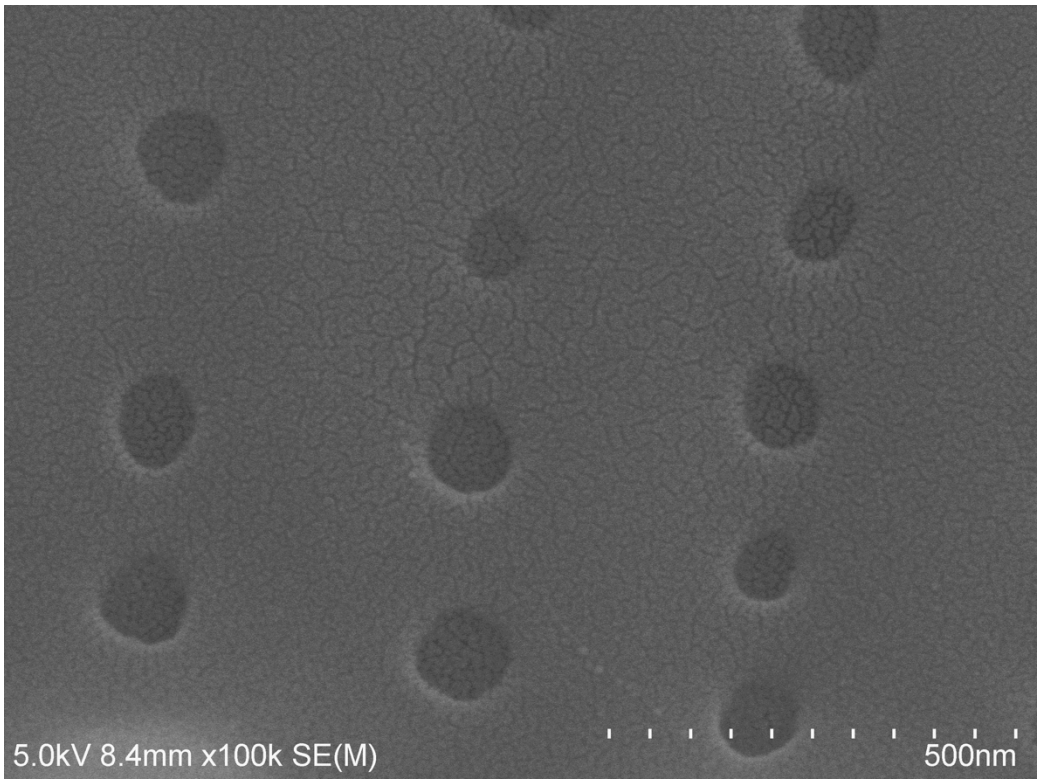
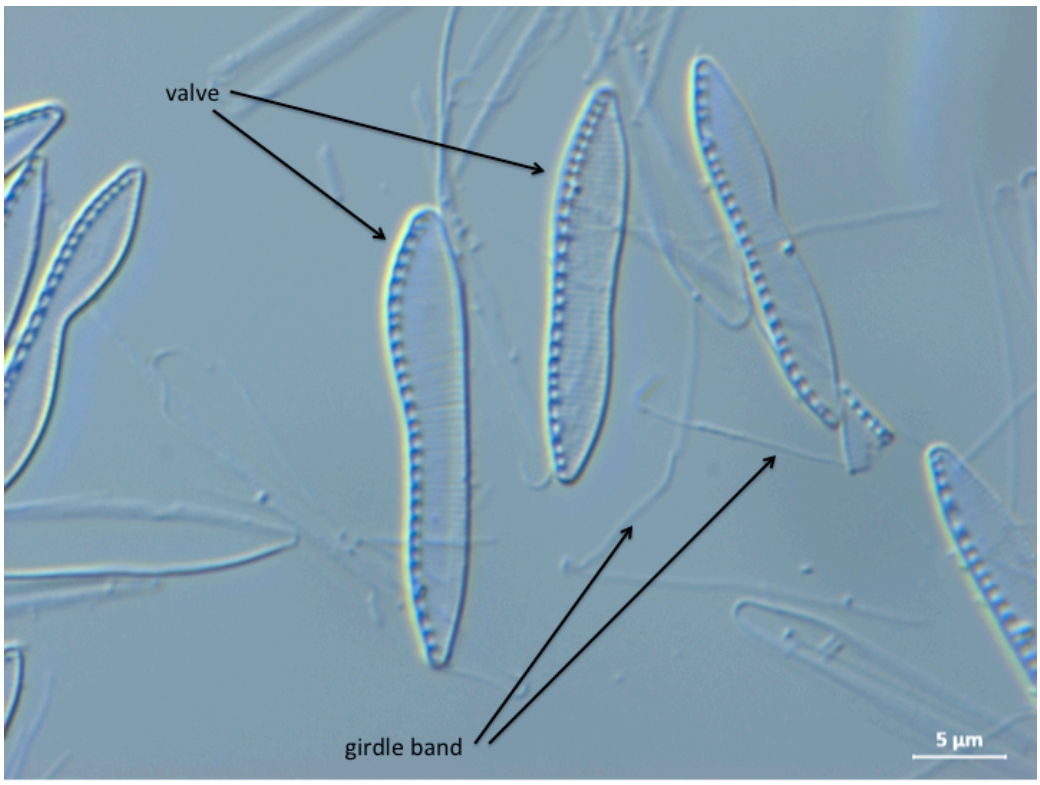


Fig. 14 GAI-216 farm sample showing primarily girdle bands and some valves (a, b). Panel c shows girdle bands only with some mineral particles. Panel d shows fully enclosed poroids indicating that this cleaning process did not dissolve the thinnest silica.

a.



b.

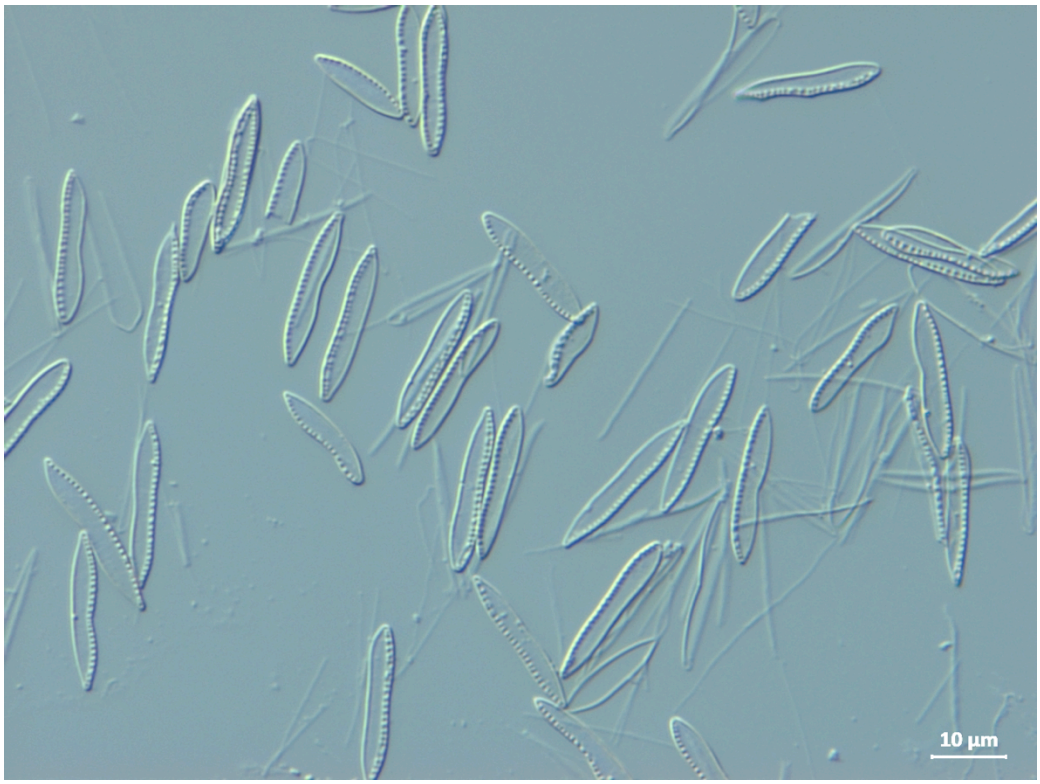


Fig. 15 GAI-216 farm sample showing valves and girdle bands.

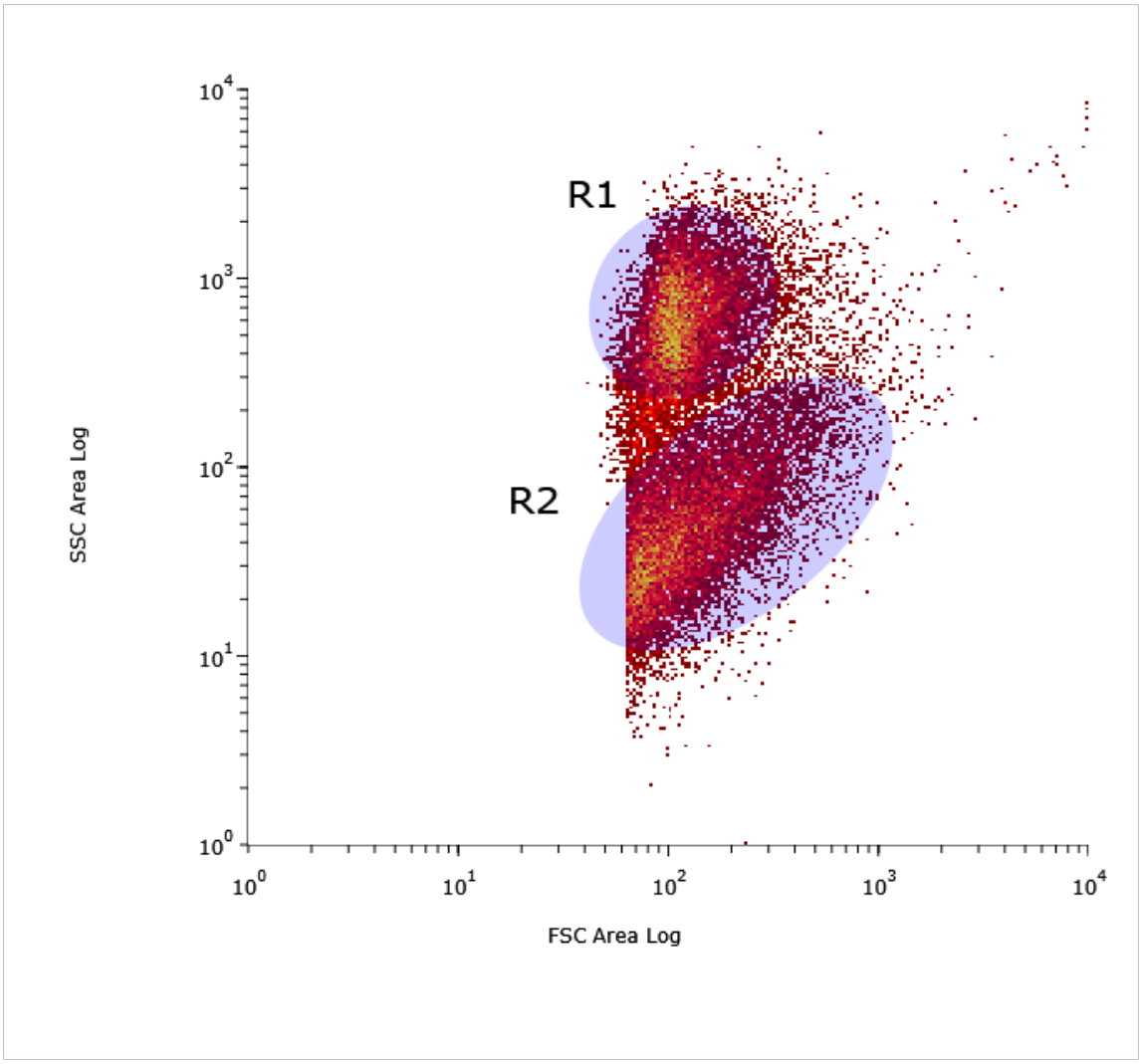
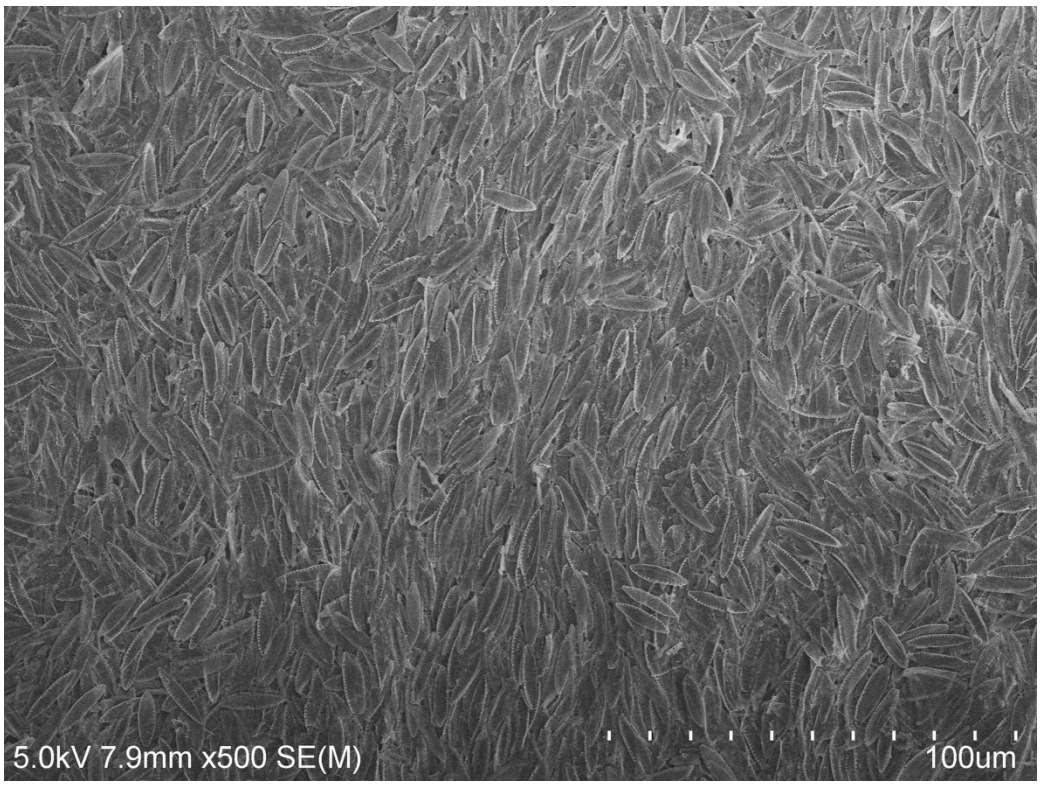


Fig. 16 Flow cytometry graph showing 2 populations of particles (R1 and R2 – ellipses in purple) examined under FSC and SSC. Regions R1 and R2 account for over 89.4% of all particles. Region R1 accounts for 39.6% of all particles and it consisted of valves and girdle bands.

GAI-205 Nitzschia sp.

GAI-205 sample from the lab was examined under the SEM and GAI-205 sample from the farm was examined under light microscope. Both samples show mostly valves and only a few girdle bands (Fig. 17 and Fig. 18). Girdle bands probably were washed out during the rinsing process.

a.



b.

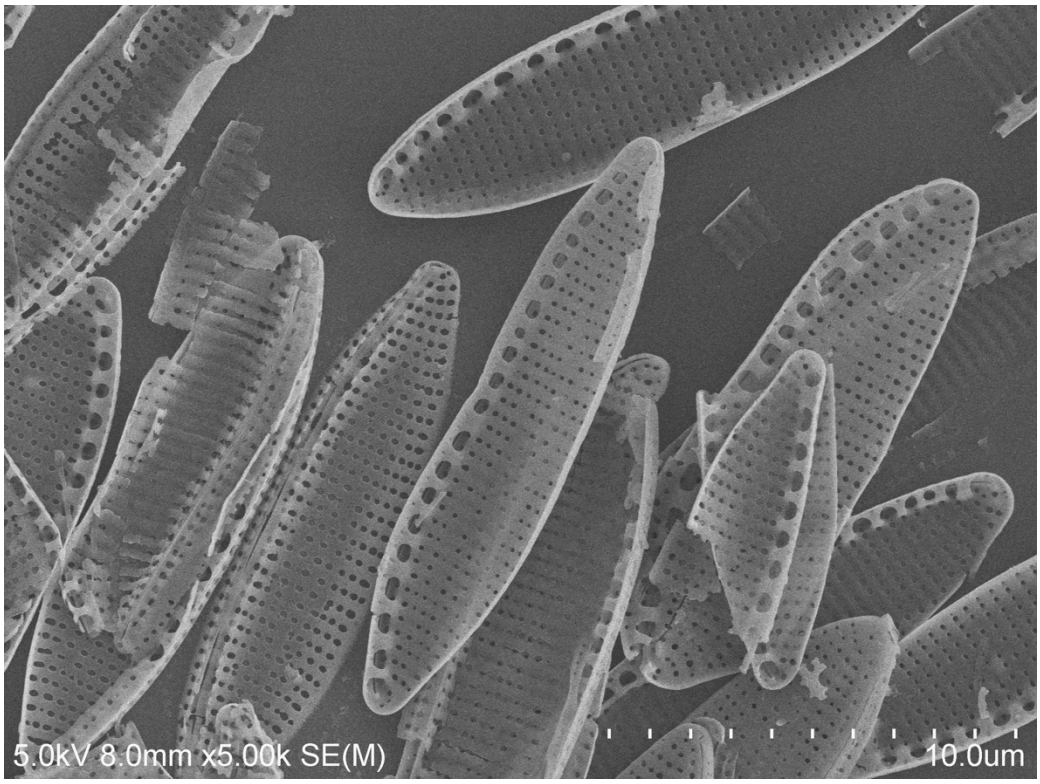
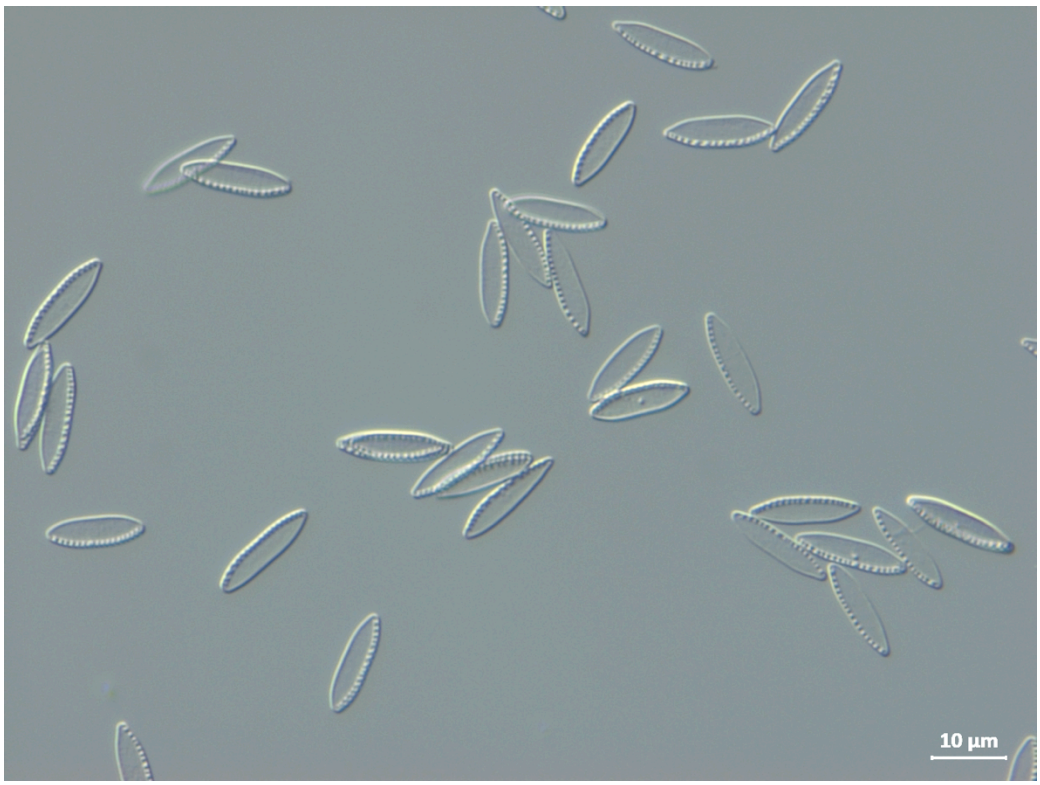


Fig. 17 GAI-205 farm sample showing mostly valves.

a.



b.

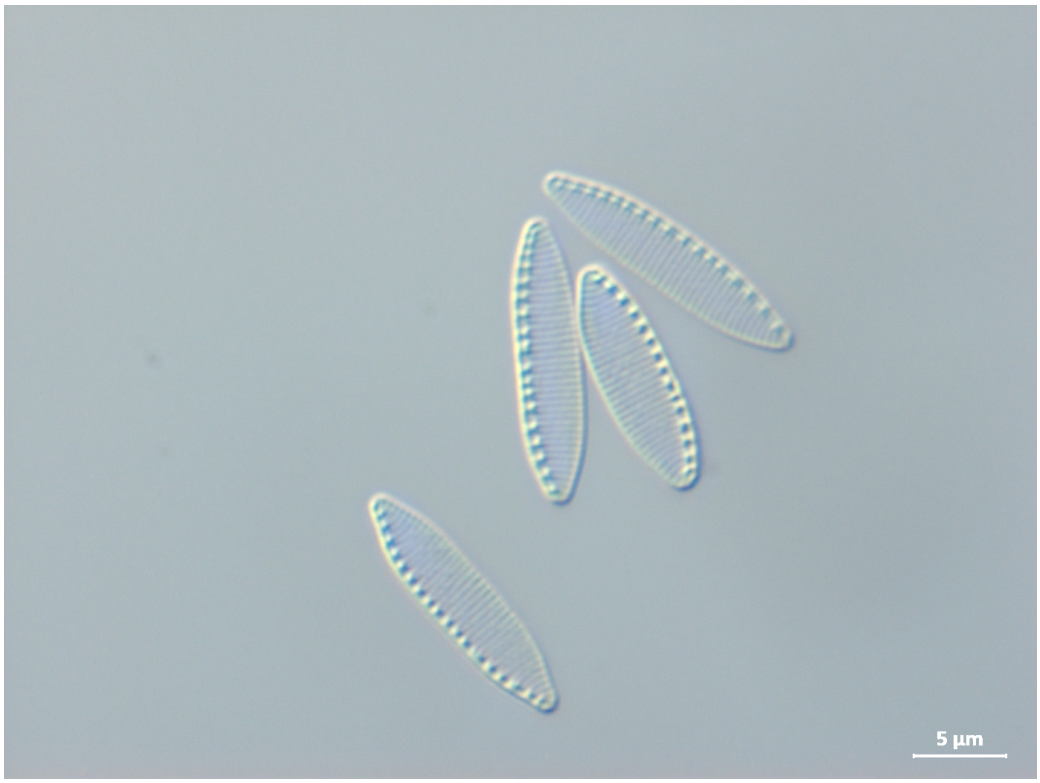
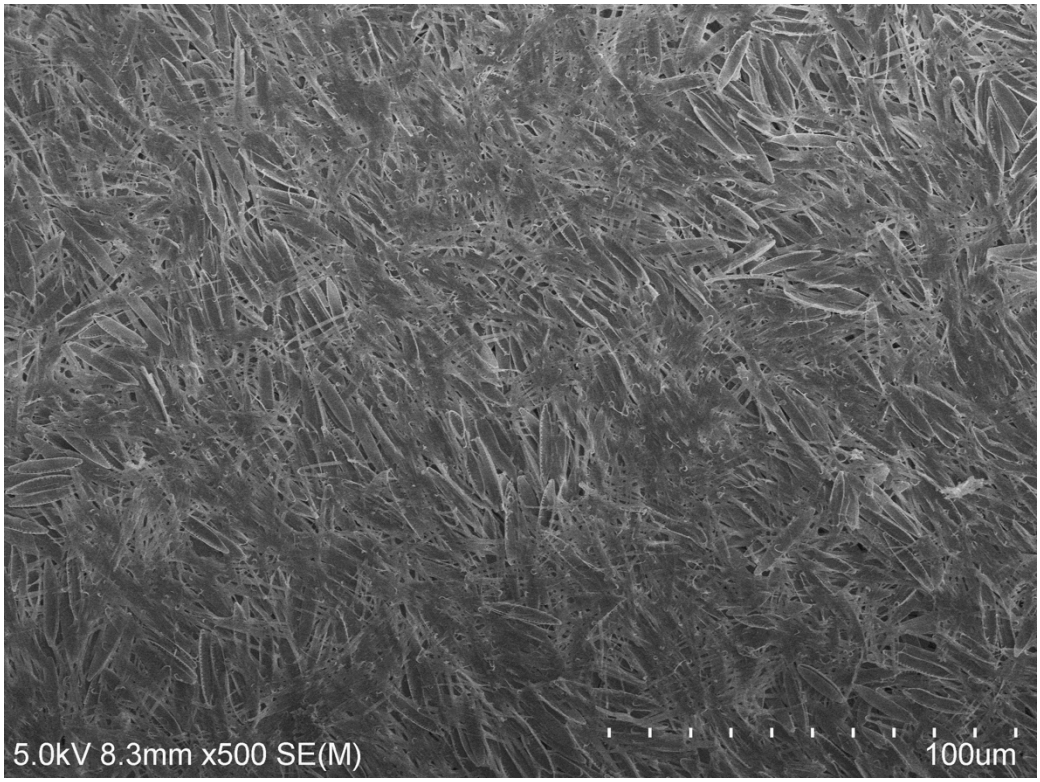


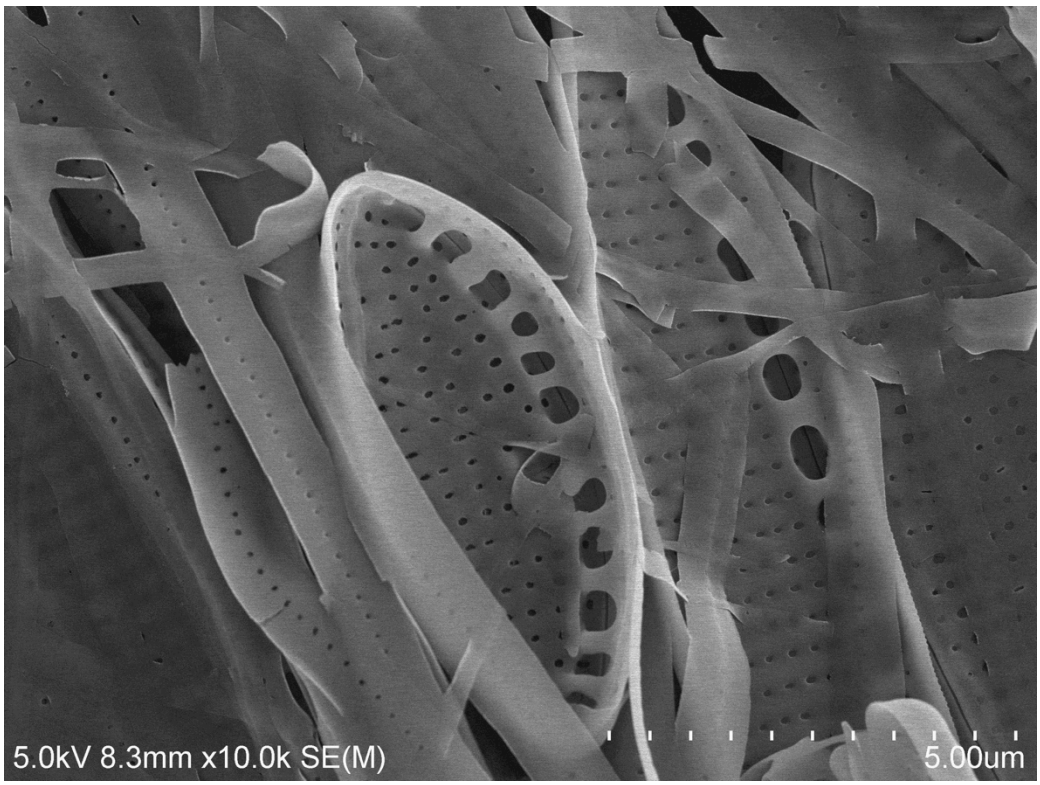
Fig. 18 GAI-205 farm sample showing valves.

GAI-229 Nitzschia sp.
GAI-229 farm and lab sample SEM images show a mix of valves and girdle bands. Material looks clean. Some of the poroids are still closed indicating gentle cleaning process (Fig. 19). GAI-229 farm images from LM show very clean material and primarily valves are visible (Fig. 20).

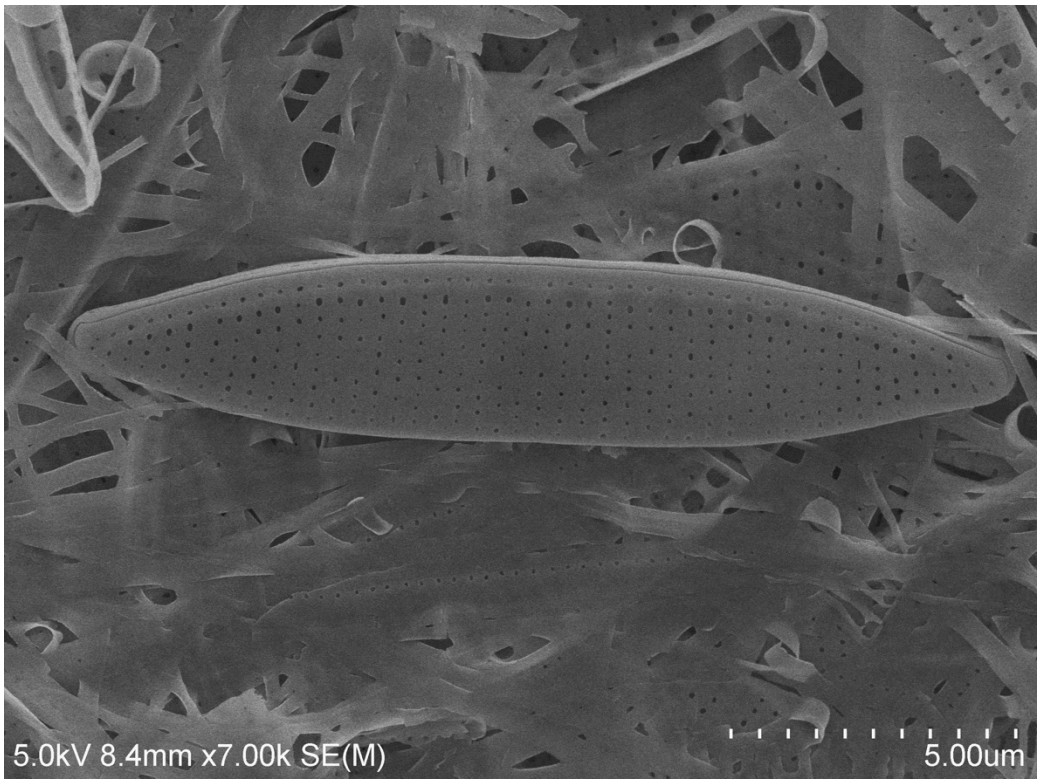
a.



b.



c.



d.

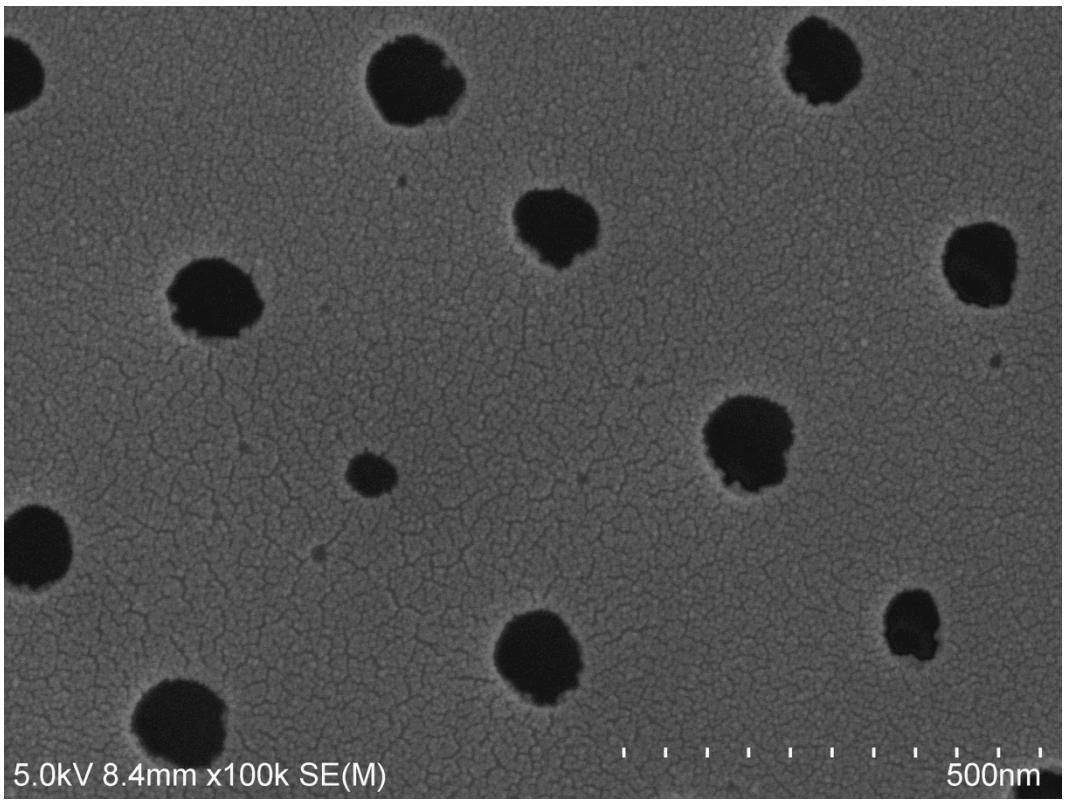
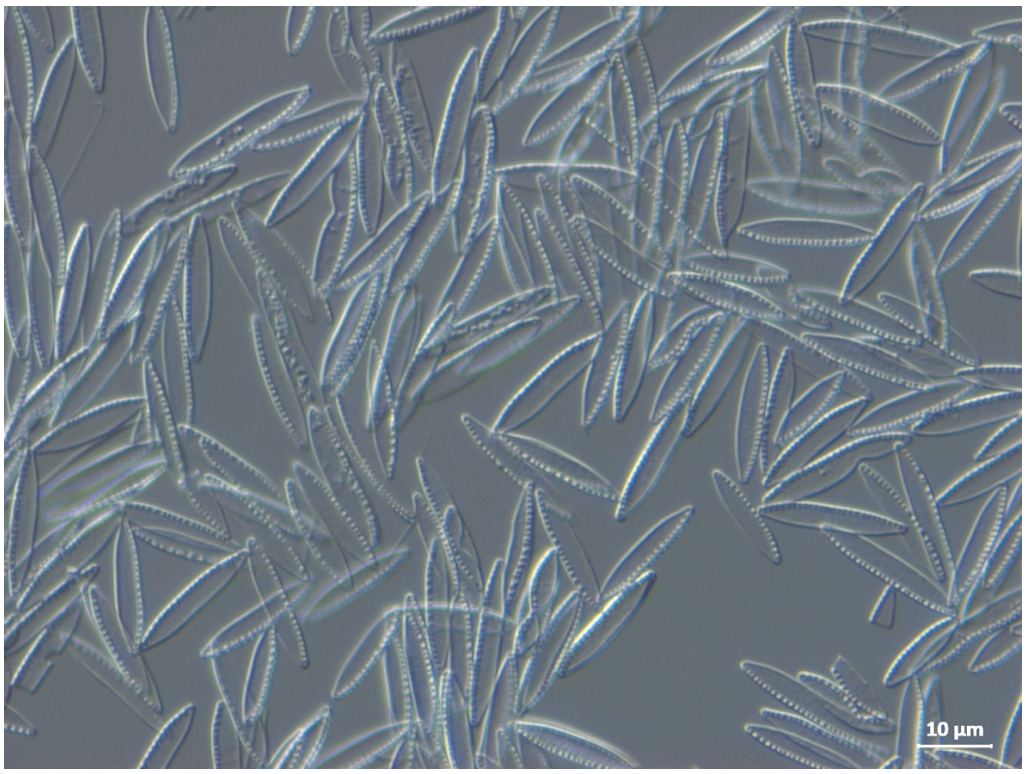


Fig. 19 GAI-229 SEM images of farm (a,b) and lab (c,d) material.

a.



b.

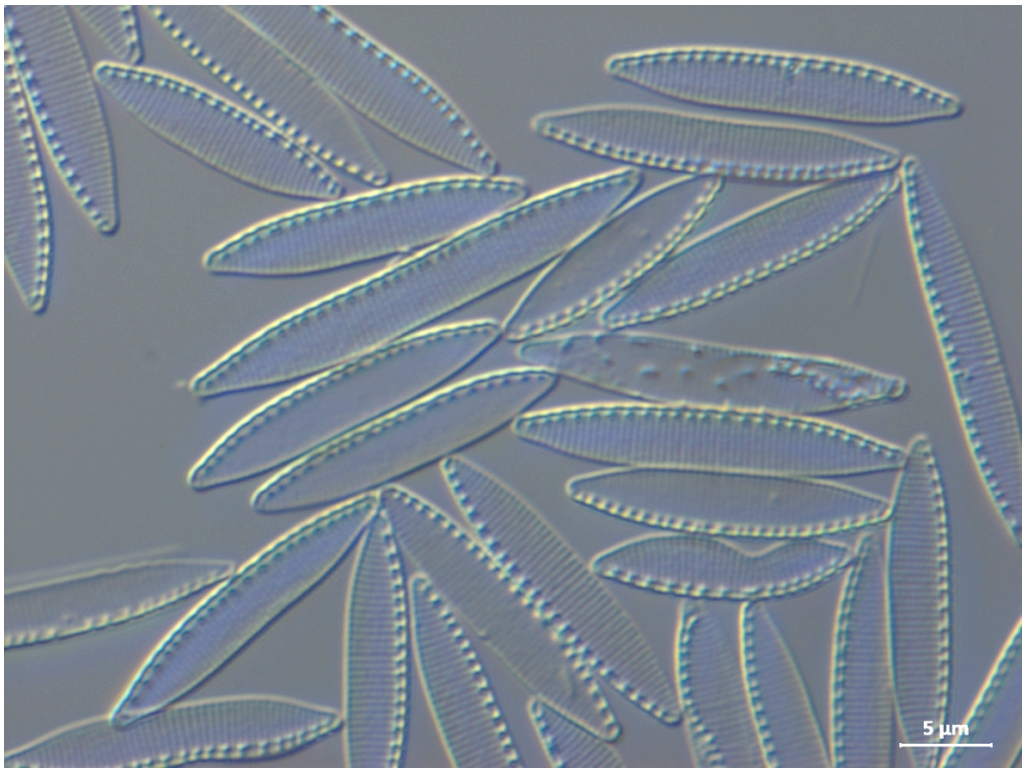
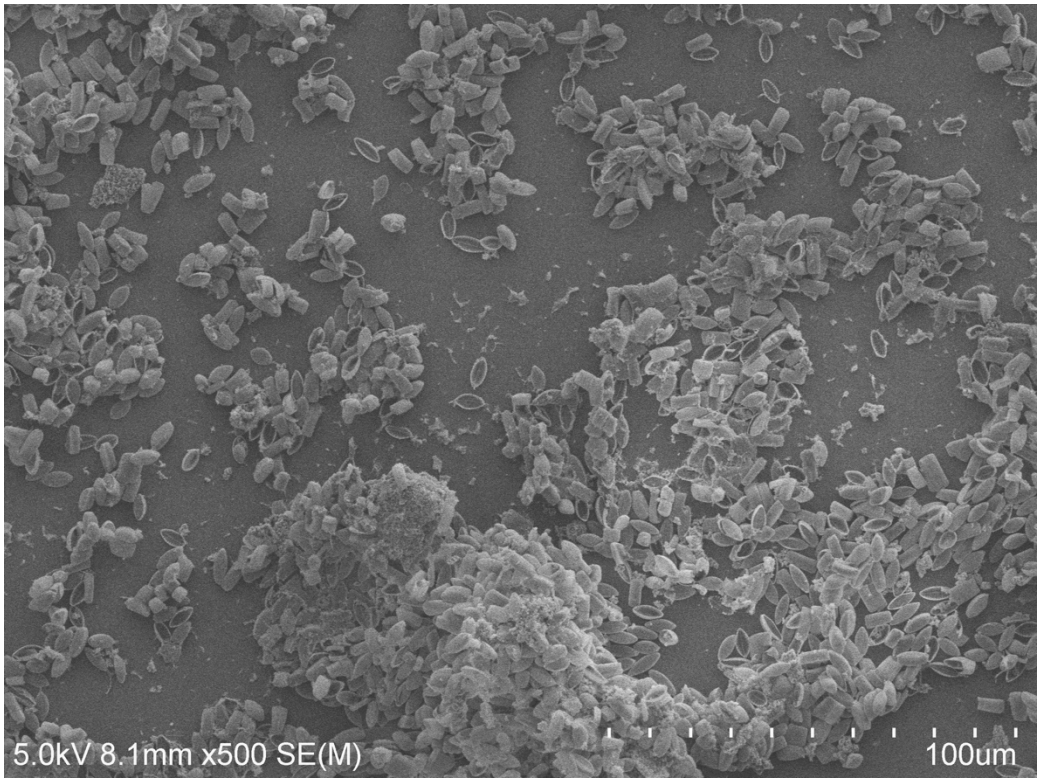


Fig. 20 GAI-229 light microscopy images of farm material.

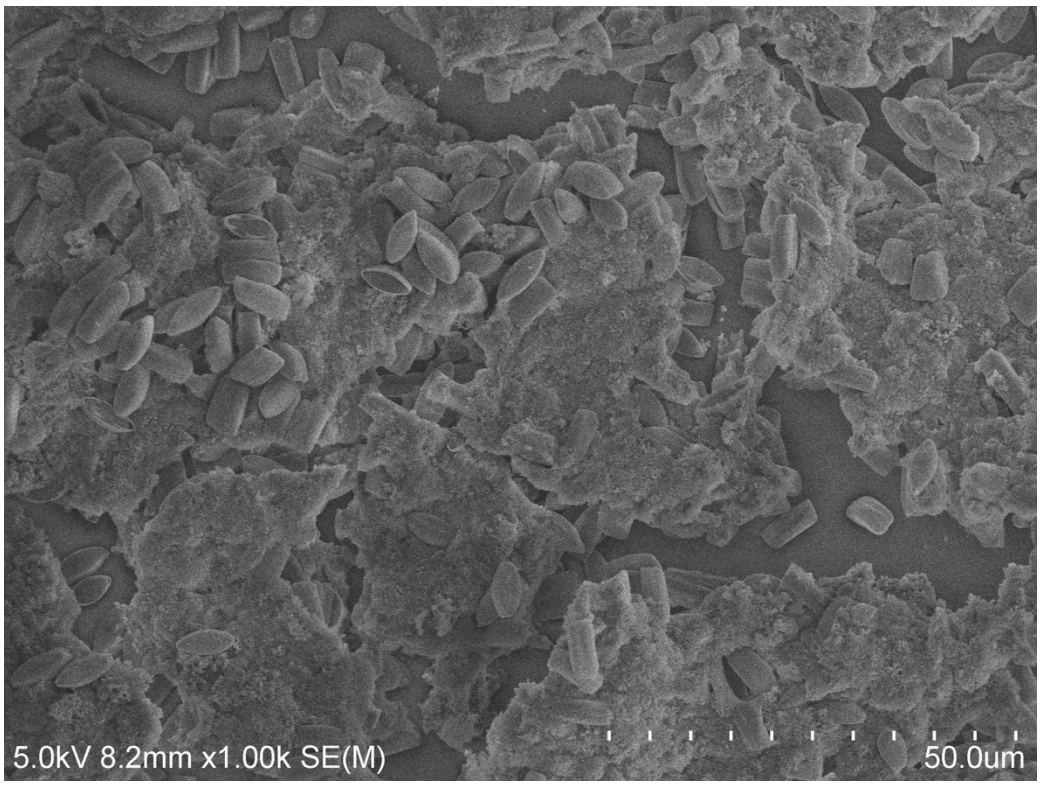
GAI-208 Navicula sp.

GAI-208 has heavily silicified cell walls. Many of the valves stayed attached together after the cleaning process. Some are separated and single valves are visible. The single girdle bands detached from the valves are very rare. GAI-208 was cultivated in marine media and when samples were dried there was a lot of precipitate visible in the material under SEM and LM (Fig. 21 and Fig. 22). Some of the precipitate was also visible in the cell wall sample suspended in water (Fig. 23). This precipitate probably formed during growth or cleaning process and was not dissolve during the rinsing process. It indicates that diatoms cultivated in seawater have to be rinsed out before cleaning process to avoid formation of precipitate. Cleaning method has to be designed for the media that algae were cultivated in.

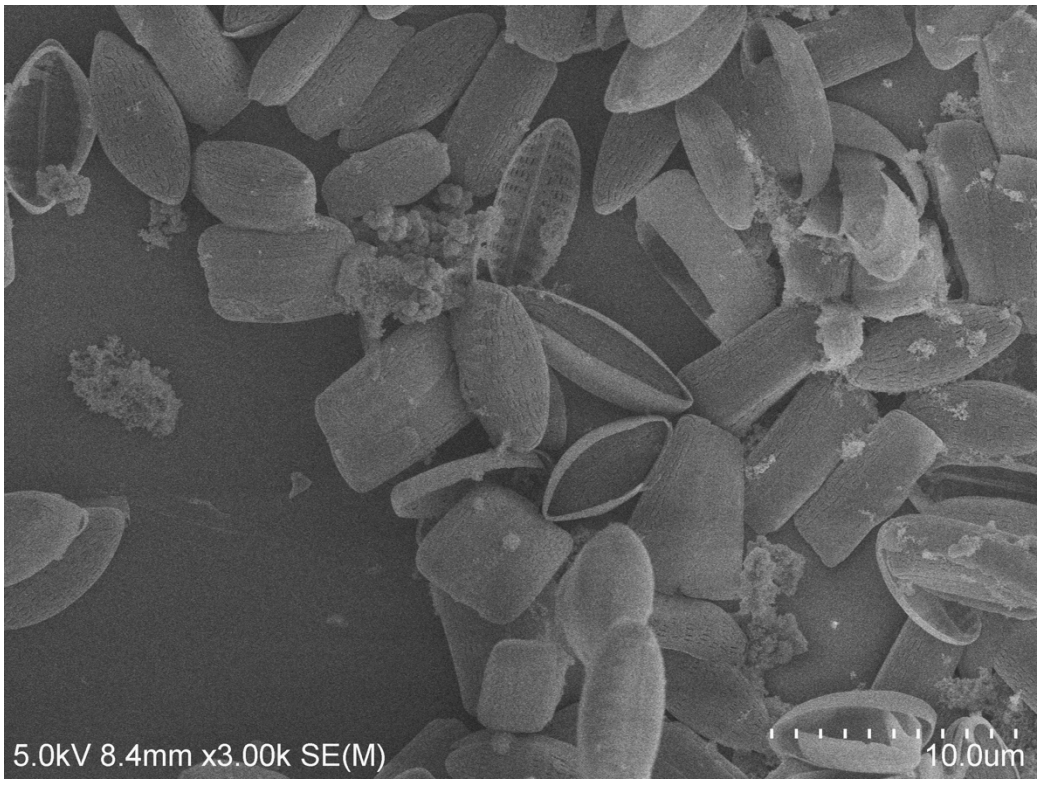
a.



b.



c.



d.

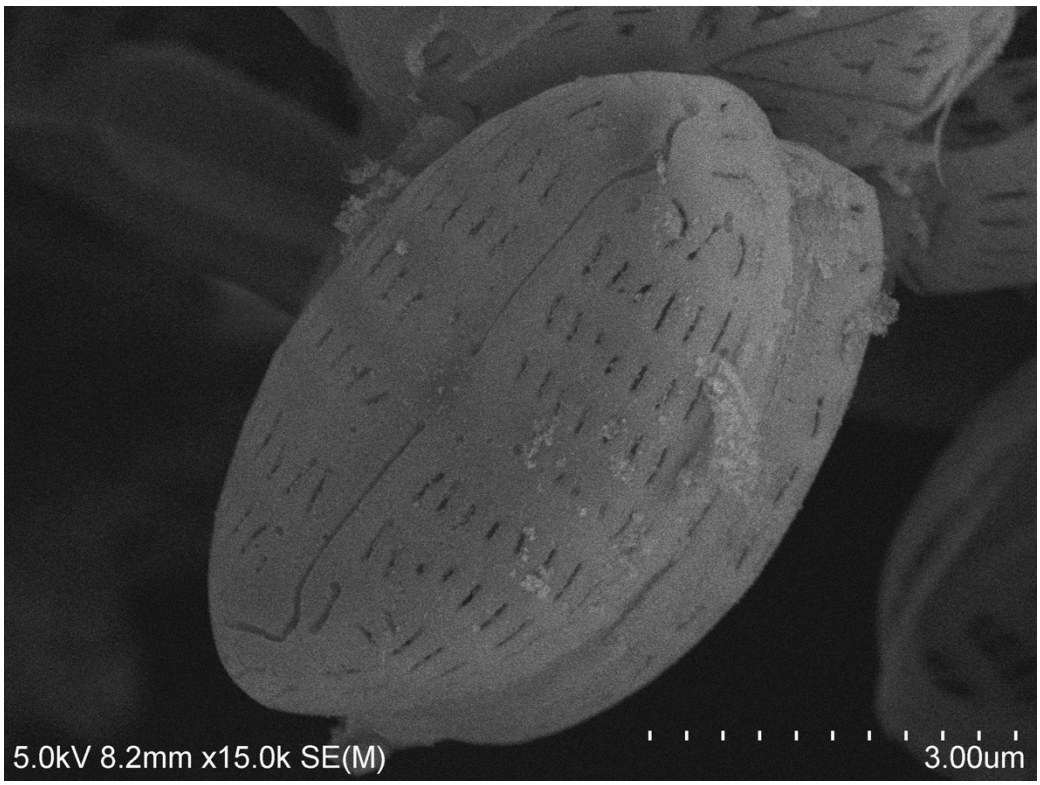
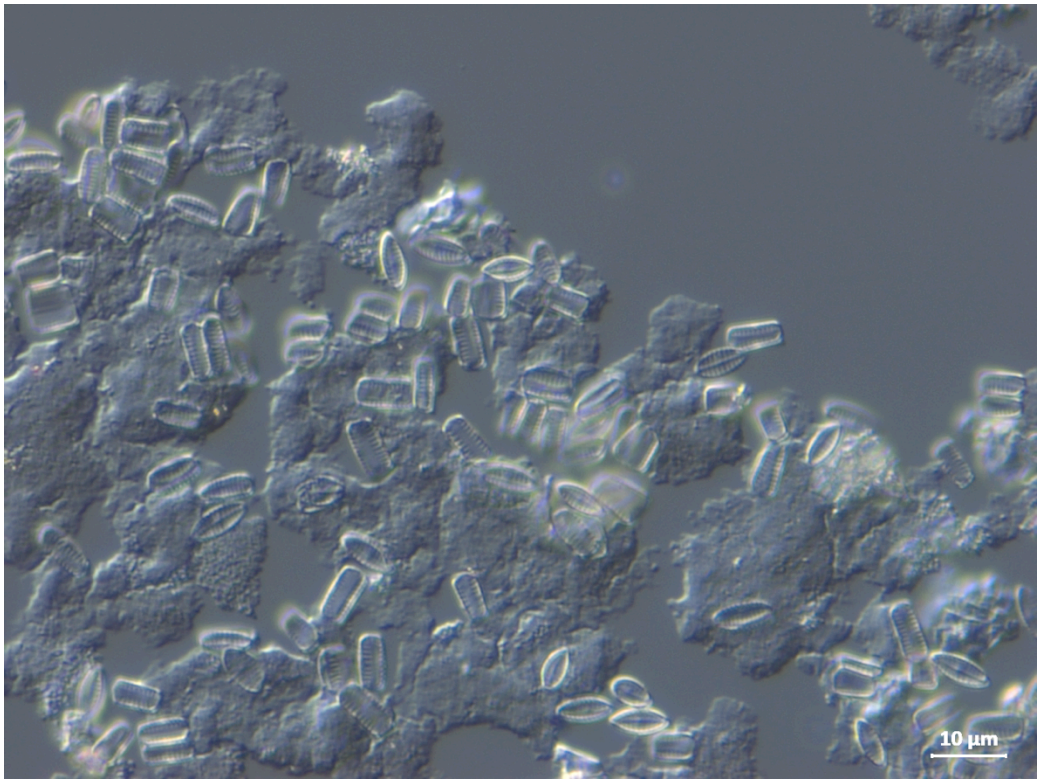


Fig. 21 GAI-208 SEM images. Samples had a lot of precipitate and there was a lot of charging while micrographs were taken. This is why images look grainy. Precipitate is visible under all magnifications.

a.



b.

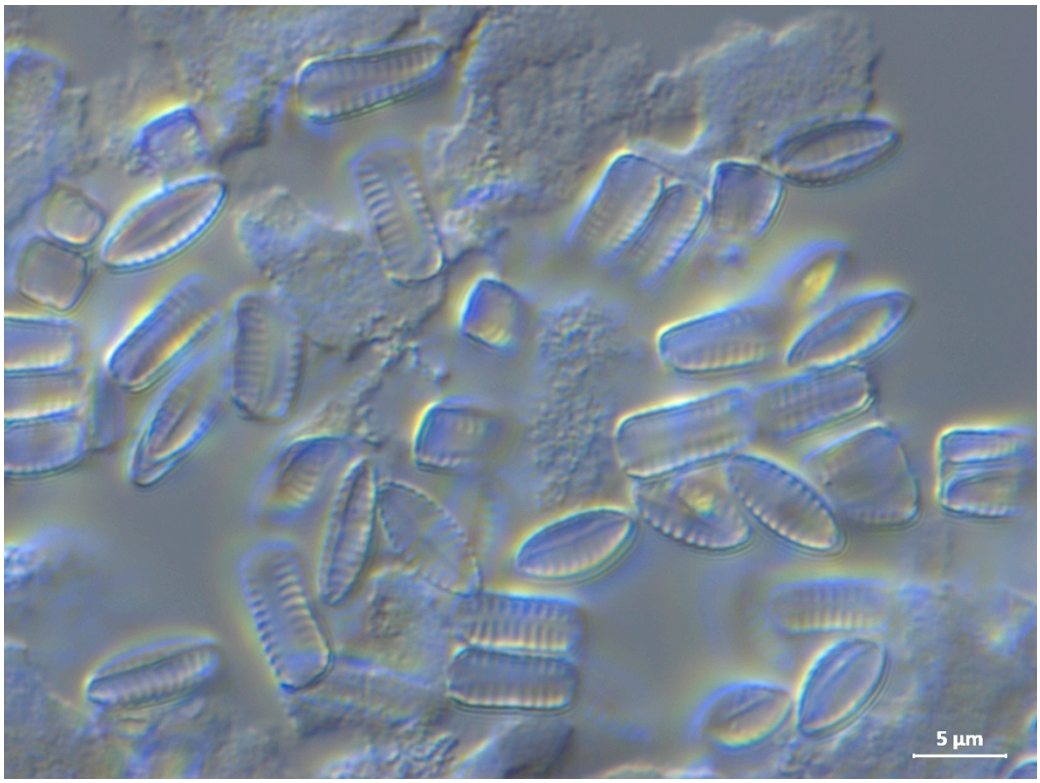


Fig. 22 GAI-208 cell walls under LM. A lot of precipitate is visible.

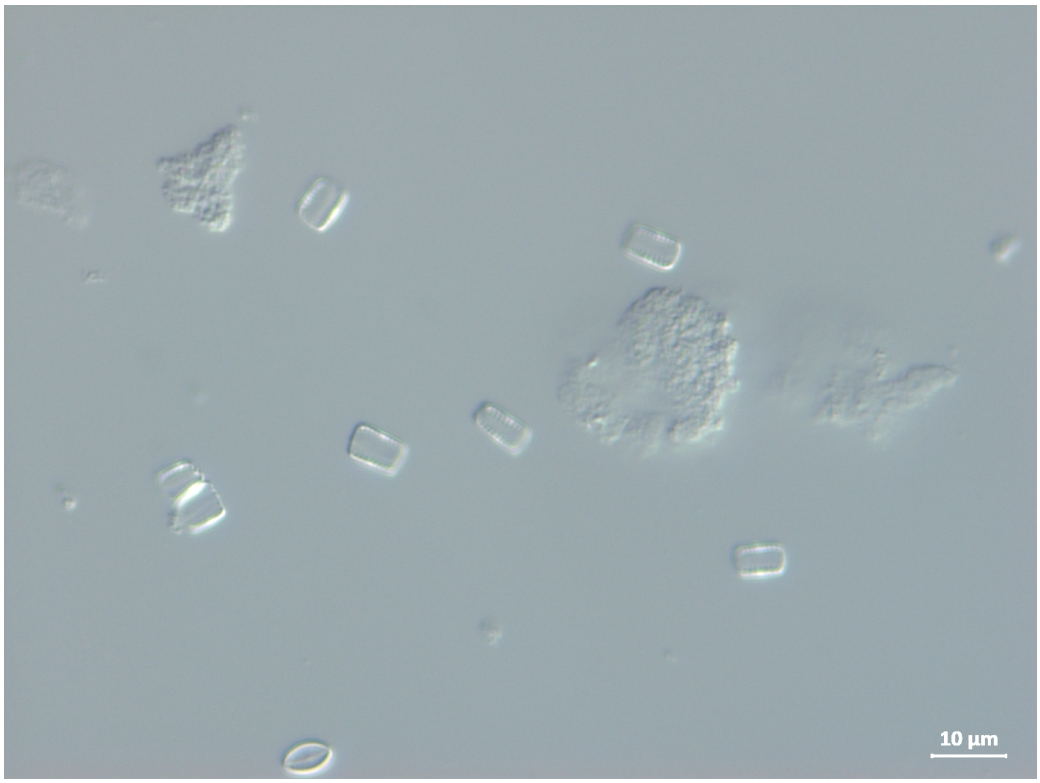
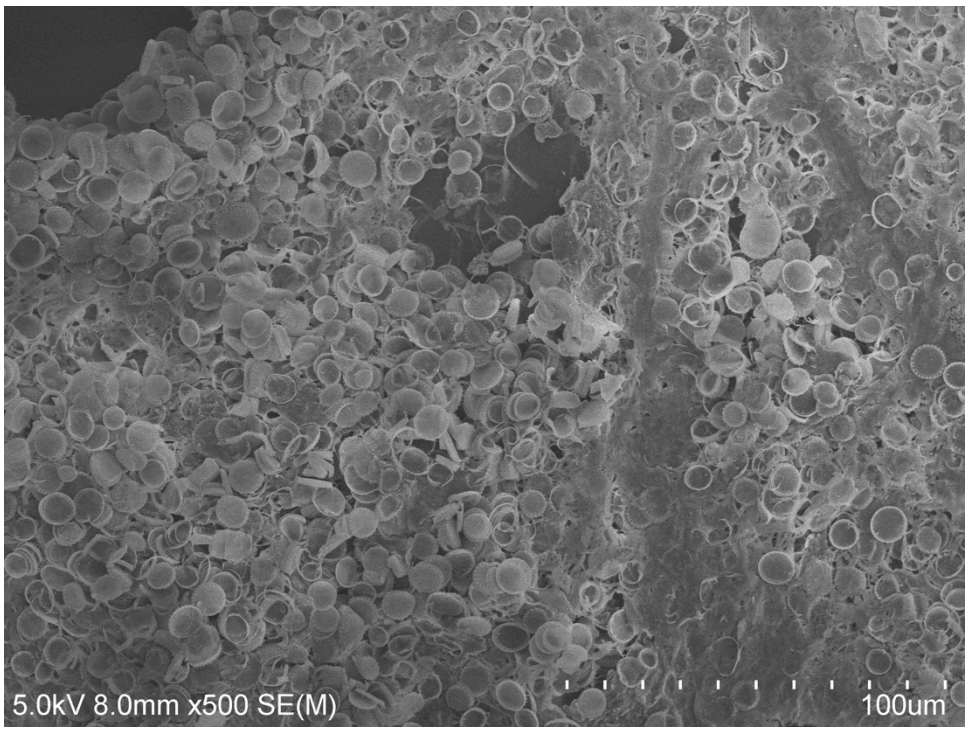


Fig. 23 GAI-208 cell walls in water showing precipitate.

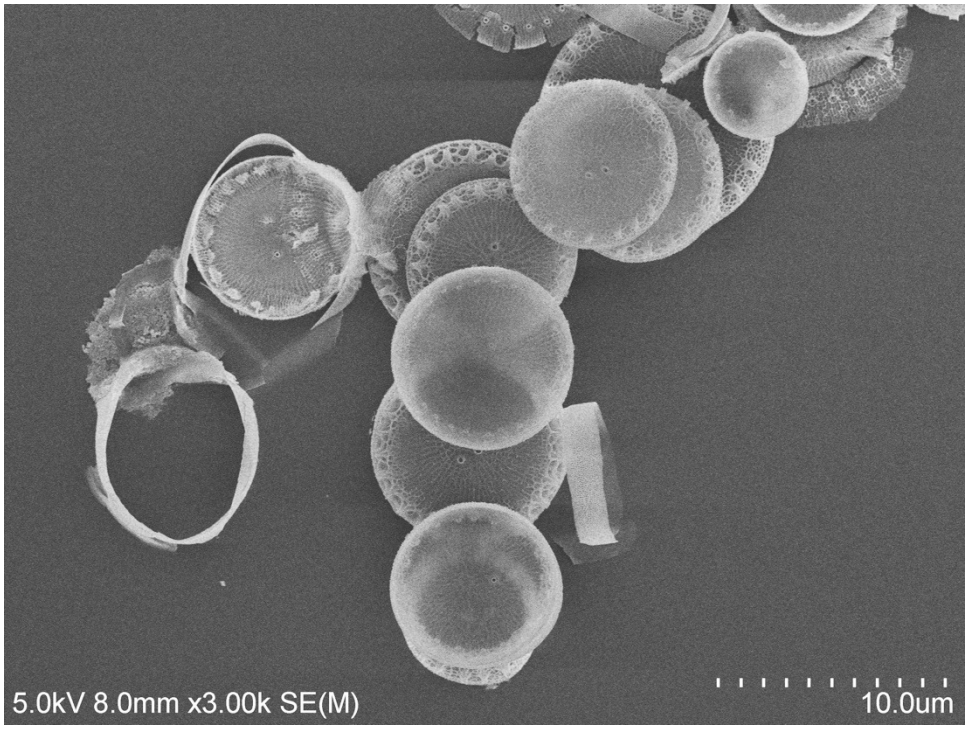
GAI-005 Thalassiosira weissflogii

GAI-005 has very intricate design of valves with many poroids that probably contribute to the large surface area of the cell walls of this species. Most of the GAI-005 valves did not separate. Frustules that separated are broken down into valves and girdle bands. Girdle bands are well visible on SEM micrographs. Since GAI-005 was cultivated in seawater media precipitate is visible on both LM and SEM micrographs (Fig. 24 and Fig. 25).

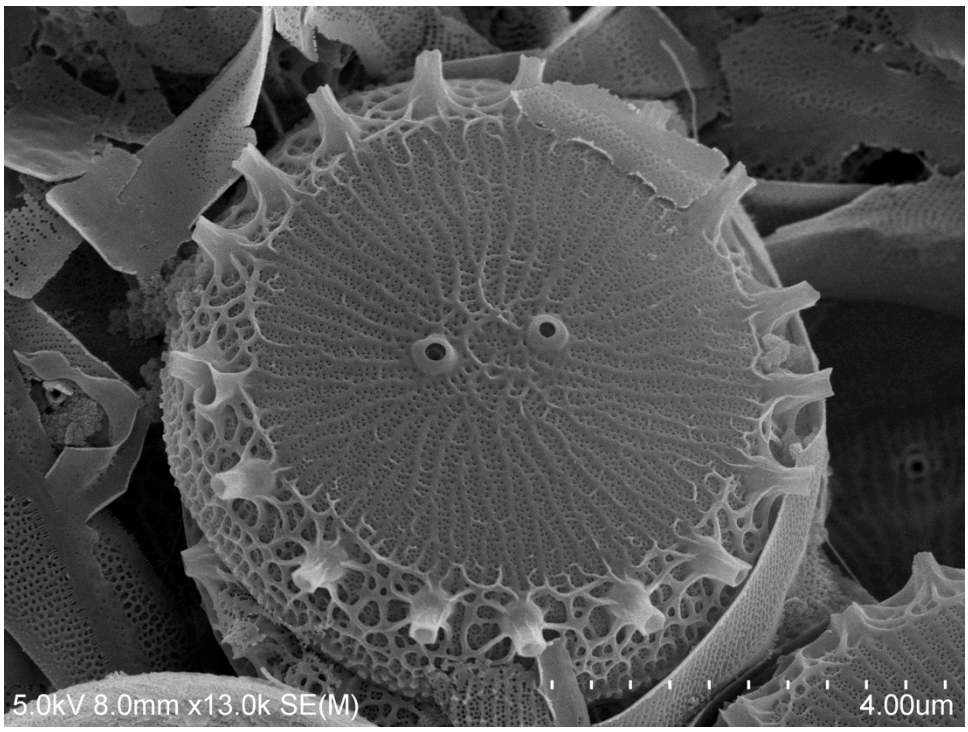
a.



b.



c.



d.

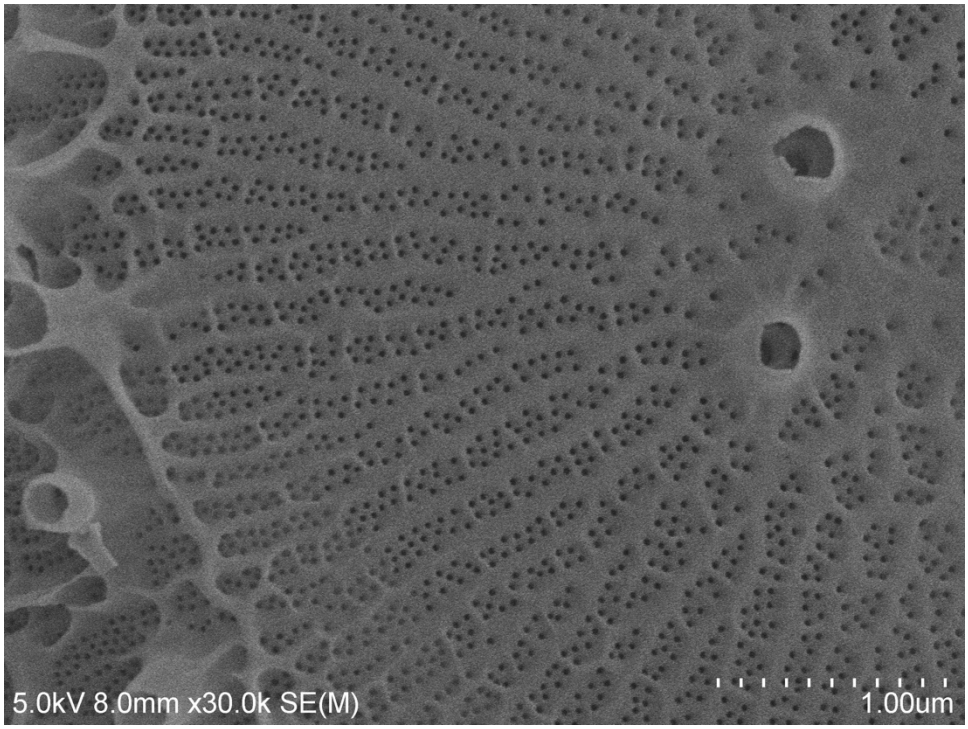
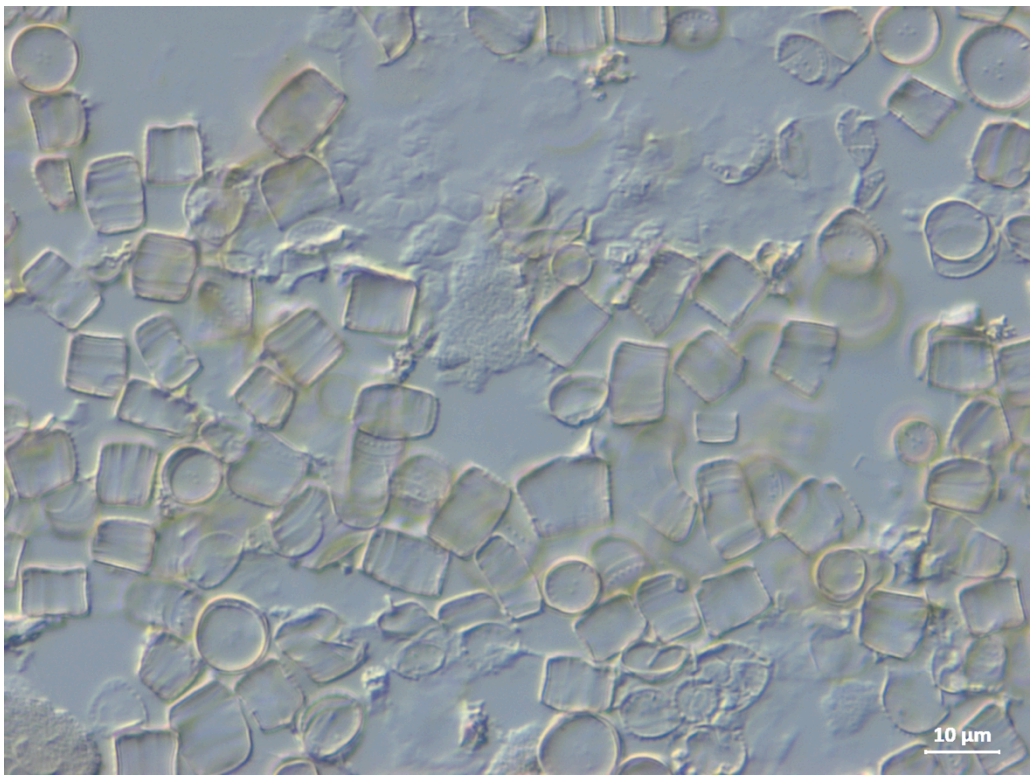


Fig. 24 SEM micrographs of GAI-005. Some valves and girdle bands are separated (a and b). Precipitate is in the form of fibrous film (a).

a.



b.

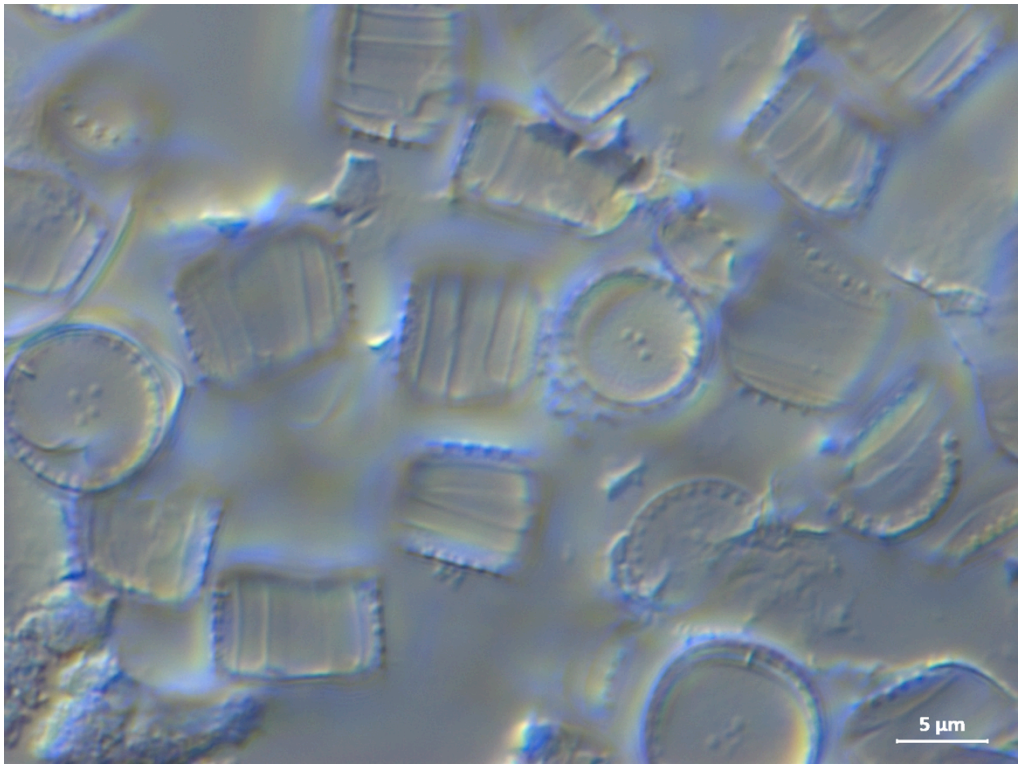
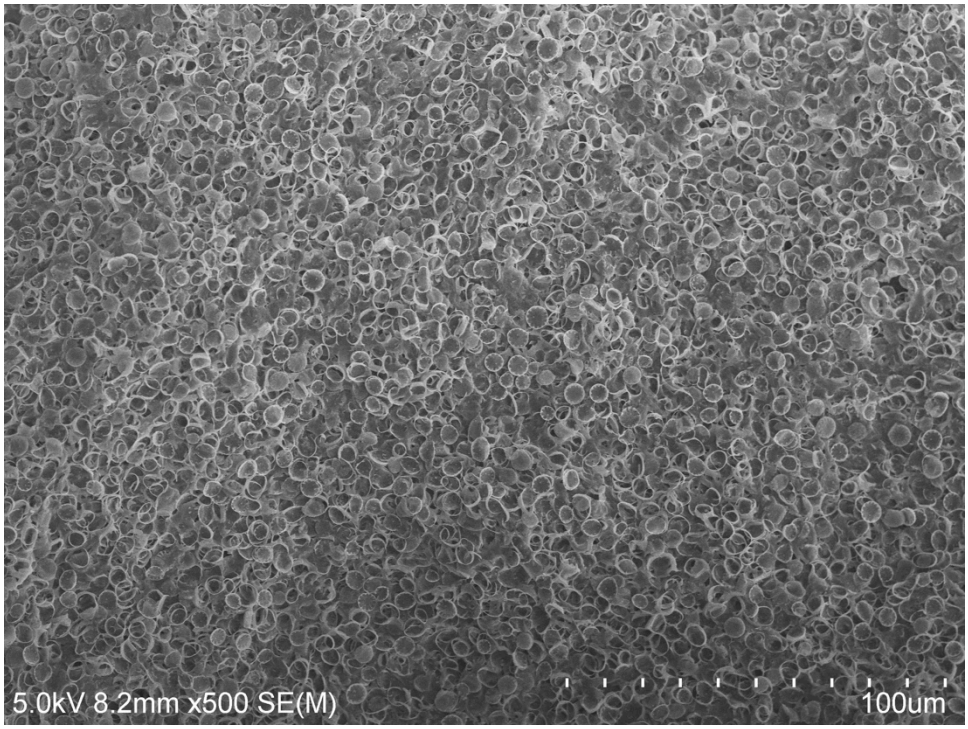


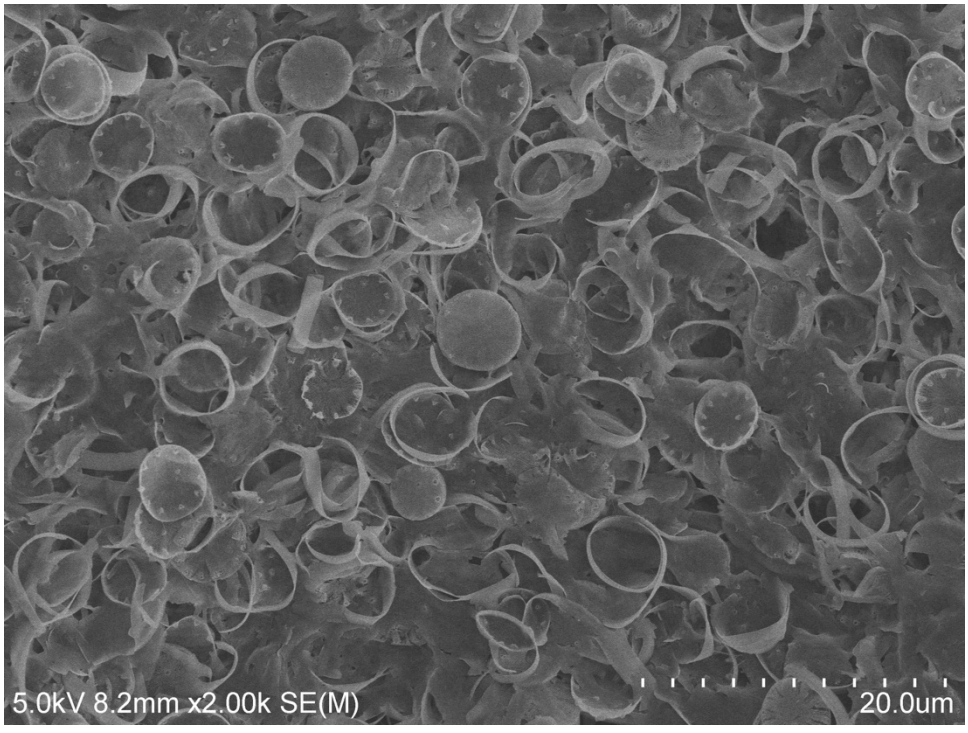
Fig. 25 GAI-005 LM micrographs showing frustules with some valves separated. Girdle bands are very fine and they are not very visible on LM micrographs. Some precipitate can be observed.

GAI-209 Cyclotella sp.
Frustules of GAI-209 separated into valves and girdle bands during processing. GAI-209 was cultivated in freshwater media and there is almost no precipitate in the sample (Fig. 26 and Fig. 27). Material looks very clean.

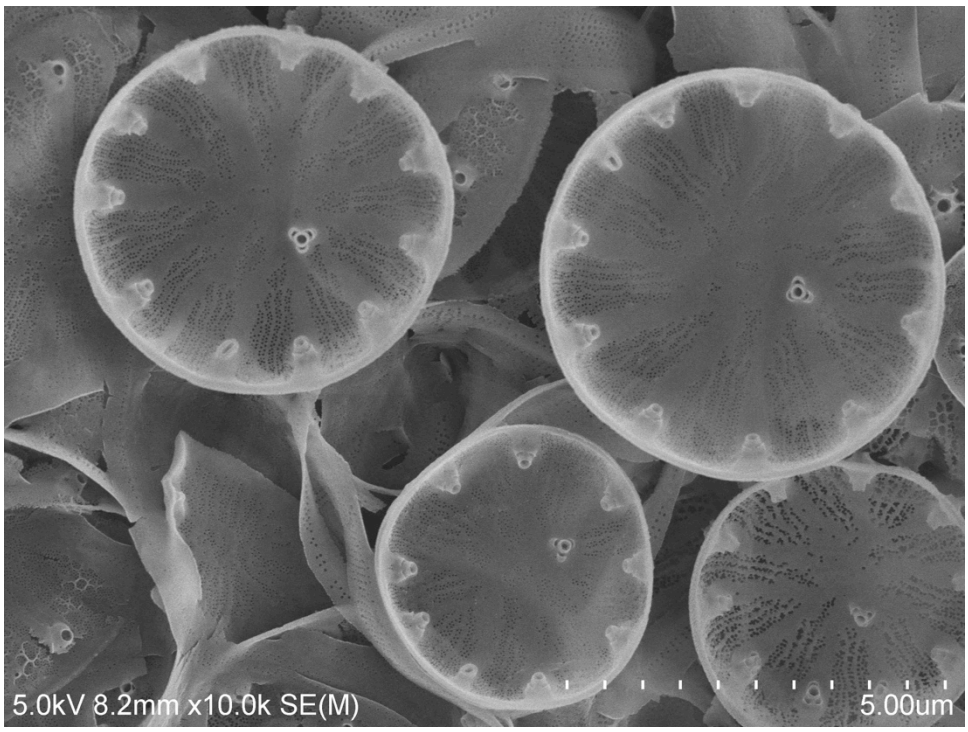
a.



b.



c.



d.

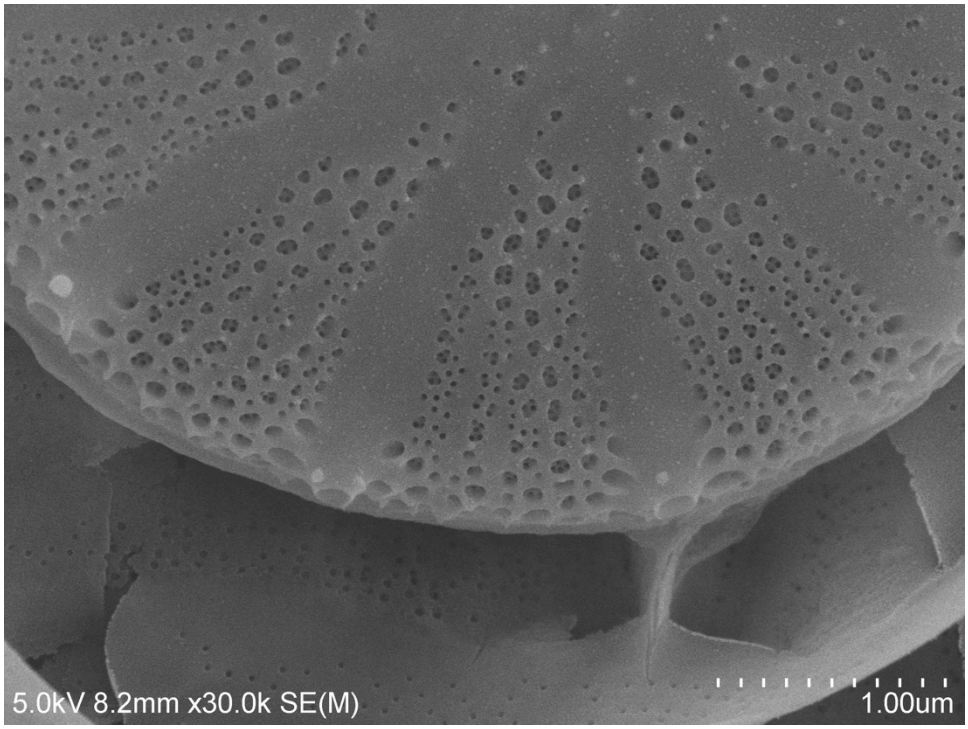
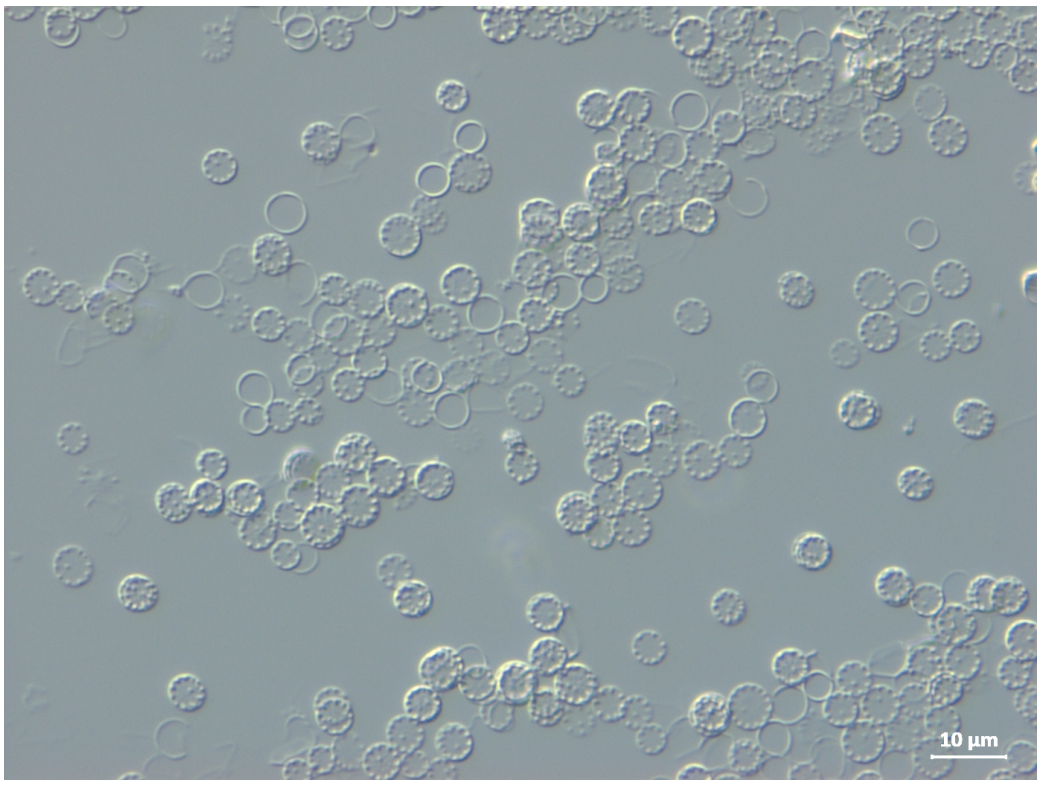


Fig. 26 GAI-209 SEM micrographs showing clean material with frustules separated into valves and girdle bands. The inside of the valves is shown on panel c.

a.



b.

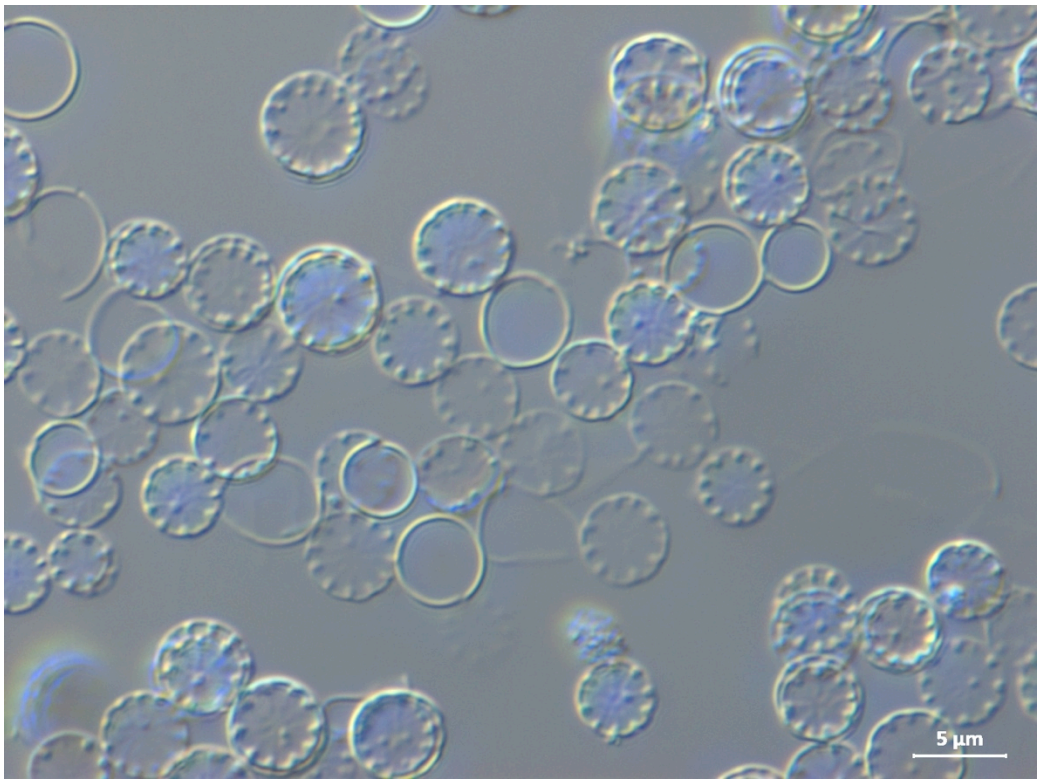
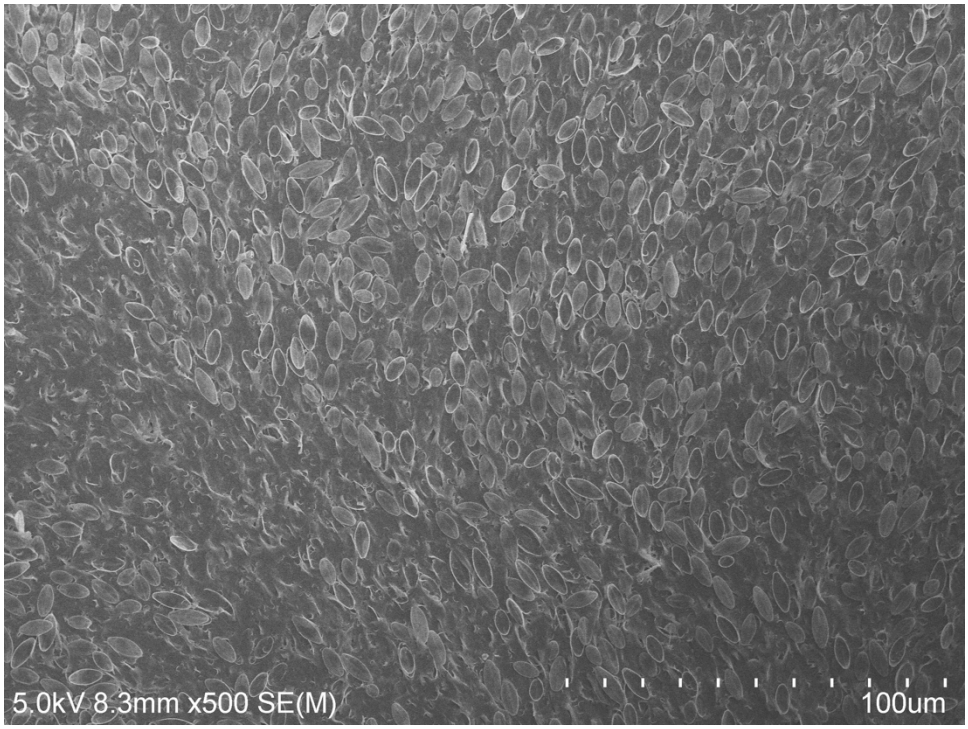


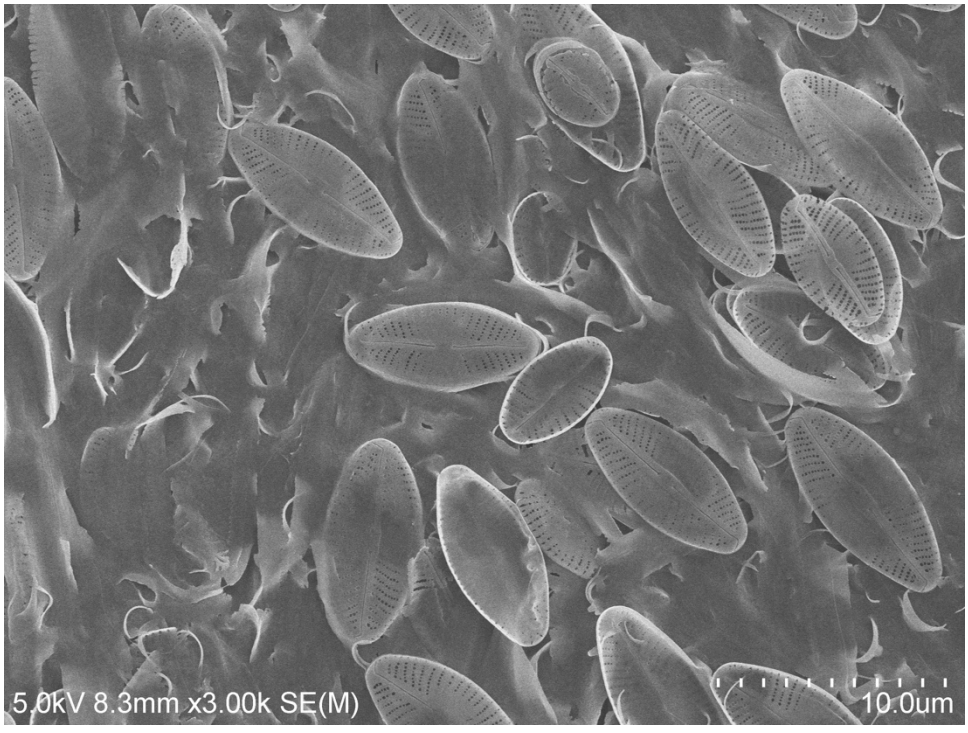
Fig. 27 GAI-209 valves and girdle bands (open circles).

GAI-215 Navicula sp.
GAI-215 frustules separated into valves and girdle bands and both are visible on SEM and LM micrographs (Fig. 28 and Fig. 29). Material was cultivated in the lab and is very clean without any precipitate. Poroids are open (Fig. 28 d)

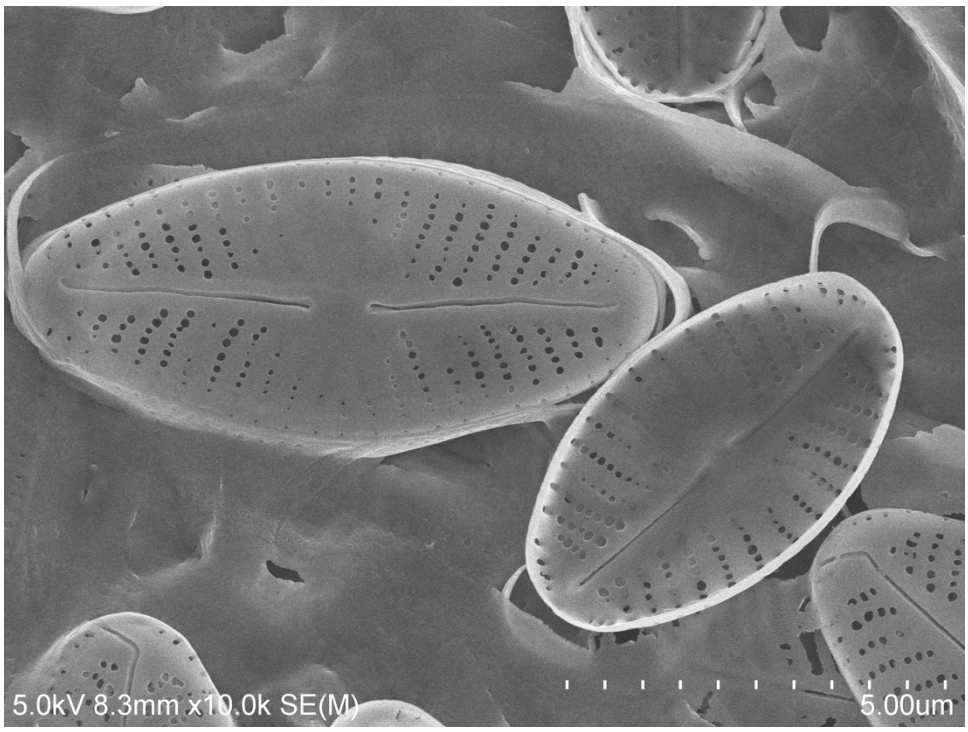
a.



b.



c.



d.

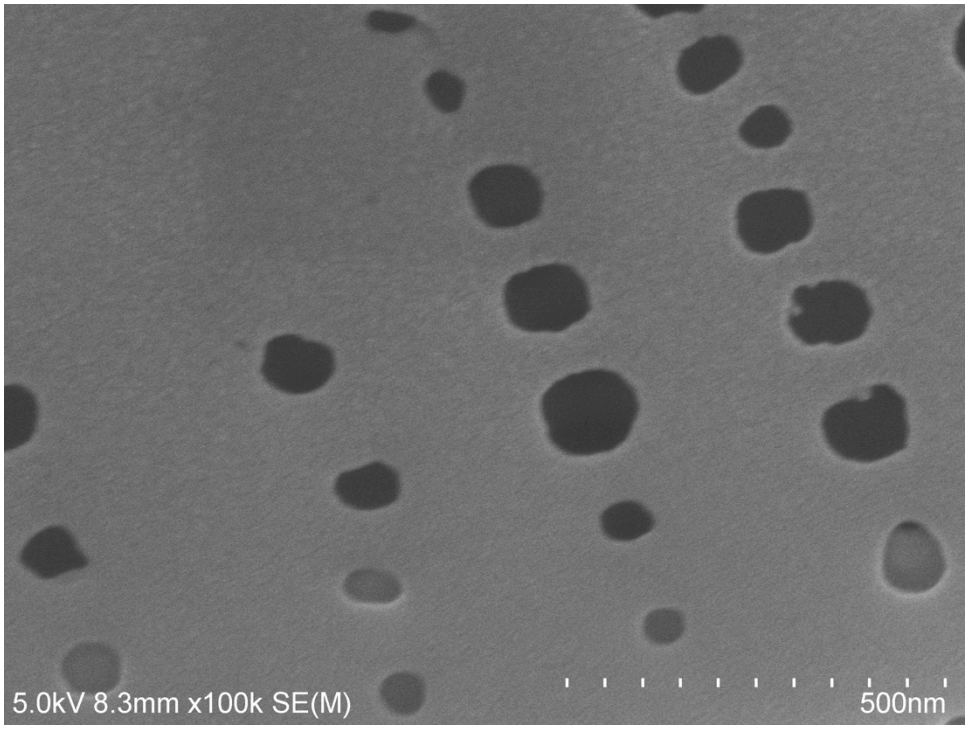
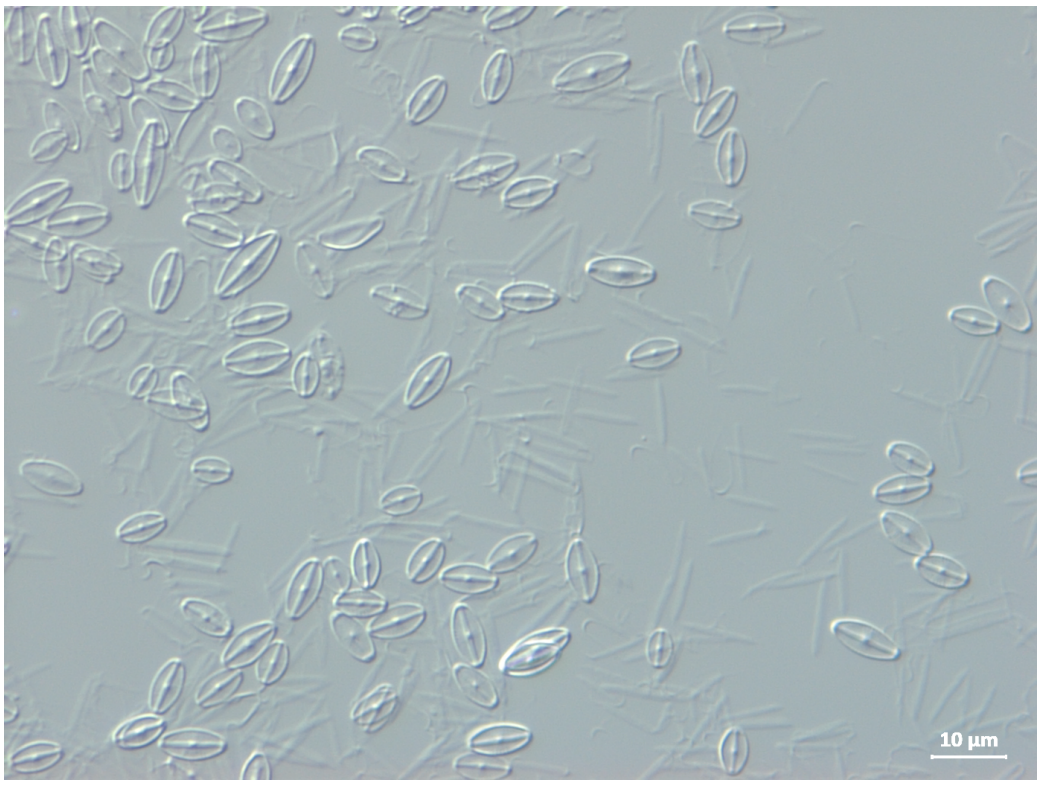


Fig. 28 GAI-215 SEM micrographs. Girdle bands are well visible on panel b and c. Open poroids are visible on panel d.

a.



b.

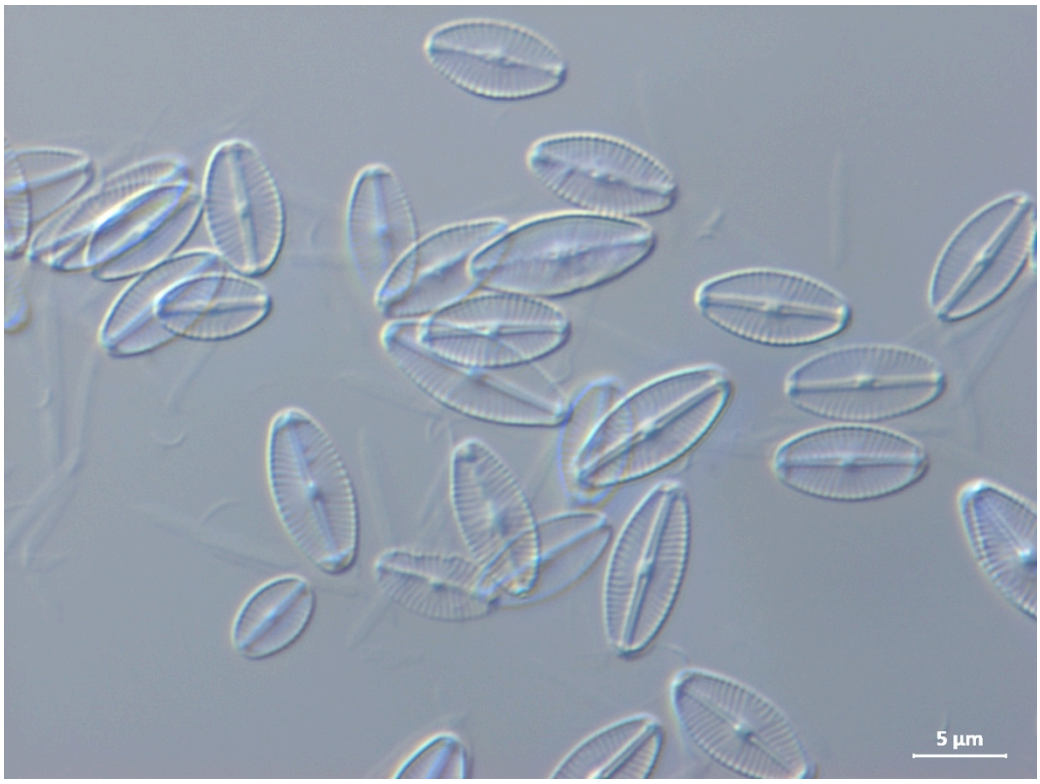


Fig. 29 GAI-215 LM micrographs showing valves and girdle bands.

Conclusions

We produce large quantities of cell walls in the open pond system at Kauai Algae Farm. Our harvesting methods work very well and did not damage the cell walls. Cleaning of cell walls needs to be designed for final cell wall application. Cleaning that produced clean cell walls also over processed the material with pores in the poroids disappearing in the process. Gentle cleaning process left the poroids but material was not as clean. It is also will take further development to scale up the lab method and to obtain uniform processing of cell walls in the large scale vessels. The next step is to find more strains of diatoms with different shapes and sizes that can be cultivated in KAF pond system and improve the cleaning process. Cell walls from diatoms cultivated at the farm have some soil particles in them but most of the particles were removed by a method of settling and sieving. Settling also separated valves and girdle bands. Further cleaning of soil particles and separation of final material into valves and girdle bands should be developed.

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Bibliography

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Appendix

Detail information on volume of culture cultivated, biomass harvested and quantities of final cell walls produced.

Species	Farm or Lab	Minimum deliverables - diatom cell walls	Harvested culture volume (L)	Total DW harvested (g)	Total AFDW harvested (g)	Description of final material	Volume of final material (L)	Weight of final material if dry (g)	DW of final material (g)	AFDW of final material (g)	Ash of final material (g)	% ash in DW	% Moisture	%DW	%AFDW	DW (g/L)	AFDW (g/L)
GAI-216	Farm	1000g	47,500	31700	23,750	Liquid	300		3902.6	124.46	3778.1	96.8				13.01	0.41
Nitzschia sp.	Farm					Liquid	300		3514.9	111.26	3403.7	96.8				11.72	0.37
GAI-205	Farm	20g	8,500	5100	3,569	Liquid	12.512		1081.7	34.00	1047.7	96.9				86.46	2.72
Nitzschia sp.	Lab					Liquid	0.061		0.38	0.06	0.32	84.1				6.30	1.00
GAI-209	Farm	10g	472	194	120	Liquid	0.129		0.79	0.02	0.76	97.2				6.09	0.17
Cyclotella sp.	Lab		80	48	34	Liquid	0.168		7.69	0.53	7.16	93.1				45.75	3.15
GAI-005	Farm	10g	673	633	243	Liquid	3.49		55.71	4.80	50.91	91.4				15.96	1.38
Thalassiosira weissflogii	Lab		120	92	54	Liquid	0.67		7.12	0.59	6.53	91.7				10.62	0.88
GAI-229	Farm	20g	621	660	372	Solid crumble		594			126.39	94.8	77.56	22.44	5.18		
Nitzschia sp.	Lab					Liquid	0.06		1.47	0.08	1.39	94.7				24.50	1.30
GAI-208	Farm	20g	500	415	250	Liquid	2.25		50.76	2.00	48.76	96.1				22.56	0.89
Navicula sp.	Lab cleaned farm sample					Liquid	0.568		34.85	1.15	33.71	96.7				61.36	2.02
	Lab					Liquid	0.02				0.10						
GAI-215	Lab	0g	40	14.76	9.96	Liquid	0.1		2.34	0.21	2.13	91.1				23.42	2.09
Navicula sp.																	