The Effect of Substrate Properties on the Attachment and Reproduction of Diatoms

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1 Abstract

The purpose of this experiment was to determine whether different properties of the substrate used would affect the amount of diatoms that attached and reproduced on the substrate. The most common type of diatom found was *Fragilaria sp.*. The properties examined were: substrate material, spacing of the glass beads substrate, and priming of the substrate with agar. To examine the effect of substrate material, four substrates were used: polyethylene plastic sheeting, aluminum screening, aluminum foil, and glass beads. The quantity of algae was similar for all substrates tested, with the exception of the glass beads. This substrate appeared to have many strings of algae on it, as compared to the mat-like growth seen on the other three substrates. It was unclear, however, whether this was because glass was a better substrate to grow algae on or whether the linear nature of the beads restricted algae to grow in strings rather than expand to the planar growth seen on other substrates. Therefore, an experiment was undertaken on whether the compactness of the glass beads would affect the amount of growth on each strand. The more spread out the strands were, the more growth there was on each strand. This seems to show that the linear nature of the glass beads may have facilitated the growth of more algae, rather than the planar nature of other substrates. This also may show a "shadowing" effect, where the strings of algae contacting each other may cause less growth than if the strands were separated. The last property analyzed was priming the substrate, in this case, plastic sheeting, with agar. The sheet, covered with a layer of agar, had more algal growth on it than the bare substrate; indication that agar allows more algae to bind, whether because of

its biologic properties, trace nutrients in it, or another factor. A study looking at a smaller substrate under the microscope appears to show that texture is probably not the reason why there was more algal growth. Further research into different attributes of the substrate, such as the agar priming or the optimal distance between linear substrates, or more controlled setups may give more insight as to the exact nature of algal attachment to substrates. This is part of a larger study as to growing and harvesting algae in the area for biofuel and bioremediation.

2 Introduction

When building boats and other underwater structures, engineers try their best to keep algae from adhering. Therefore, a fair amount of effort has been made to determine how algae attach and what they attach to, so that this attachment can be inhibited. Diatoms are some of the most common biofouling agents. They are photosynthetic single-celled organisms with a silica cell wall which fits together like a box. To stay afloat, they have a droplet of oil inside. Most diatoms attach to substrates both passively and actively (Wustman, et al. 1997) and they usually find the substrate just by passing by (Weatherbee, et al. 1998). If the substrate is hydrophobic, the diatom can temporarily attach passively using its preexisting surface coating. The diatom can also attach actively by secreting new extracellular matrix (ECM) polymers to facilitate adherence (Wustman, et al. 1997). These polymers are typically secreted through a slit, termed a raphe, or an apical pore field in the cell wall, termed a frustule. The ECM polymers, also known as the exopolymeric substaces (EPS) can then also facilitate a more permanent connection.

There are four major phases of adhesion for diatoms. First, the cells orient themselves so that their raphe is next to the substrate, particularly if that substrate is hydrophilic (Wang, et al. 1997). Next, the diatom stops moving and makes small pads so that it can affix itself to the substrate. The diatom then makes a shaft which connects itself to the pads just produced. This shaft can often be highly structured, flexible, and many layered (Wang, et al. 1997). These first three steps generally occur within the first two days. Lastly, the cell expands and divides. Each division occurs on the opposite side to the substrate, so eventually this will make a filamentous colony (Wang, et al. 1997). The average time it takes for the cell to divide is 24 hours.

The EPS is generally made up of sulfated polymers. In *A. longipes*, for example, there is an inner

sulfated core oriented perpendicular to shaft elongation surrounded by three outer layers oriented parallel to shaft elongation (Wustman, et al. 1997). These stalks are often covered in an organic sheath and are hydrophobic. Although some characteristics change between species, the hydrophobicity and organic sheath are often conserved (Wustman, et al. 1997). Since the EPS is composed of many stalks laid on top of each other, the outer layer first attaches, and then each of the stalks inside attaches to each other (Weatherbee, et al. 1998). Much is still not known, however, about the exact mechanism of sensing the substrate and of performing the necessary actions for attachment.

This attachment strategy could affect the ability of diatoms to attach to different substrates, and it appears to do so. Diatoms overall tend to prefer hydrophobic substrates and reject substrates that corrode easily or are not as biologically inert. These differences, while often statistically significant, did not show very large differences between the growth of diatoms or other organisms studied. Also the amount of diatom growth was not largely affected by these substrate characteristics. Many individual studies have been done on various materials to determine what algae may attach to and which are the best and worst.

Most substrate substances studied did not disintegrate over time. This makes sense, because if one is growing algae to be harvested, one would not wish to keep remaking the substrate. One study, however, suggests that biodegradable substrates are the best (Bobbie, et al. 1978). In Bobbie, et al.'s study, they looked at using plastic pine needles and organic pine needles for growing estuarine algae. Although the plastic pine needles did grow a fair amount of algae, they never did as well as the organic pine needles, which were heavily covered by the third week (Bobbie, et al. 1978). This situation, however, would not be useful for our study since we needed the substrate to maintain its integrity over time. To test this early on, burlap was used as a substrate, but this fell apart and did not show any increased amount of algae. Algae's growth many be affected by whether or not other organisms are already present or by the speed of current. Peterson and Stevenson's 1989 study looked at whether more diatoms would grow in fast currents or conditioned tiles, those with non-photosynthetic organisms already attached, versus slow currents or unconditioned tiles. More diatoms always grew in slower currents, and the differences in growth between conditioned and unconditioned tiles were not distinct (Peterson and Stevenson 1989). Therefore, diatoms' growth is not affected by the presence of bacteria and other organisms at the start of the growth period.

Corrosive metals tend be the worst for diatoms' adherence. In Marszalek, et al.'s 1979 study, glass and metal substrates were dipped into subtropical seawater to see what they would grow. The metals used were: stainless steel 304, 60/40 copper-zinc brass, 90/10 copper-nickel, and 70/30 copper-nickel. Glass and stainless

steel had the most organisms and started to grow diatoms and filamentous algae by the fifteenth day, with these groups becoming the most dominant by week three (Marszalek, et al. 1979). Over time, however, the stainless steel corroded and the abundance and diversity of organisms on it decreased. The copper-nickel alloys supported a layer of diatoms and fungi by week three, but the amount was highly variable. Any layers on top of the diatoms and fungi easily fell off (Marszalek, et al. 1979). In Sekar, et al.'s 2004 study, copper alloys, such as copper, aluminum brass, and admiralty brass, did not support as many green algae, diatoms, or cyanobacteria as hydrophobic or hydrophilic substrates. The best substrates for attachment were titanium, stainless steel, Perspex, and glass for all three species. Attachment was also better on rougher surfaces and those with a basic pH (Sekar, et al. 2004). This study was done in Petri dishes with coupons. These Petri dishes were shaken at 40 rpm so that this attachment had to be more permanent if it was to survive.

Surface wettability and tension has often been implicated in the ability of diatoms to adhere. Finlay, et al. in 2002 studied the adherence of *Amphora*, a genus of diatoms, and *Enteromorpha*, a genus of green algae, to alkane thiolates on gold, which were constructed to have different wettabilities. The wettability of a surface generally correlates to its hydrophobicity or hydrophilicity. *Enteromorpha* adhered the strongest to hydrophilic surfaces, but had more, albeit weaker, attachment to hydrophobic surfaces. *Amphora* adhered more strongly to hydrophobic surfaces, which have a higher wettability, but did not show a difference in total number attached, whether strongly or weakly, due to surface wettability (Finlay, et al. 2002). Surface tension can also affect the attachment of both diatoms and bacteria. Becker studied in 1996 the attachment strength and EPS production of both *Pseudomonas*, a bacteria, and *Amphora coffaeformis*, a diatom. The substrates used had surface tensions between 19 and 64.5 mN/m. The best attachment strength was seen on those with higher surface tensions, such as polycarbonate and glass (about 80-85 percent for both) (Becker 1996). The strongest adhesion and greatest EPS production was also on polycarbonate. The substrates with lower surface tensions did not show high EPS production, but still had diatoms growing on them (Becker 1996). Therefore, there may be other mechanisms for attachment beyond just EPS production.

Surface roughness has also been analyzed for its contribution to diatoms' ability to affix to materials. Most studies of roughness are performed using rocks and ceramic. In Bergey, et al.'s 2010 study, they looked at the colonization of *Didymospheria*, a genus of diatom, on various types of rock. More diatoms colonized sandstone, which is rougher, than shale and more colonized those stones with biofilms already on them (Bergey, et al. 2010). Clifford, et al. in 1992 used clay tiles in Rocky Mountain streams to determine whether rough or smooth tiles would grow more algae and macroinvertebrates. Both the number

of macroinvertebrates and the chlorophyll A content increased on the rougher clay tiles after the first four days (Clifford, et al. 1992). In Scardino, et al.'s study, however, no real difference between rough or smooth surface colonizations was found. A microtextured matrix with various sizes was made with a laser and *Amphora* along with other organisms were allowed to colonize the matrix. Attachment was reduced in those surfaces with a texture size below that of the organism, but increased in smooth surfaces or those with multiple attachment points for the diatom (Scardino, et al. 2008). These differences, however, were not significant. Therefore, the authors stated that a smoother surface should be used since there was no difference in attachment and smoother surfaces would make it easier to harvest the diatoms (Scardino, et al. 2008).

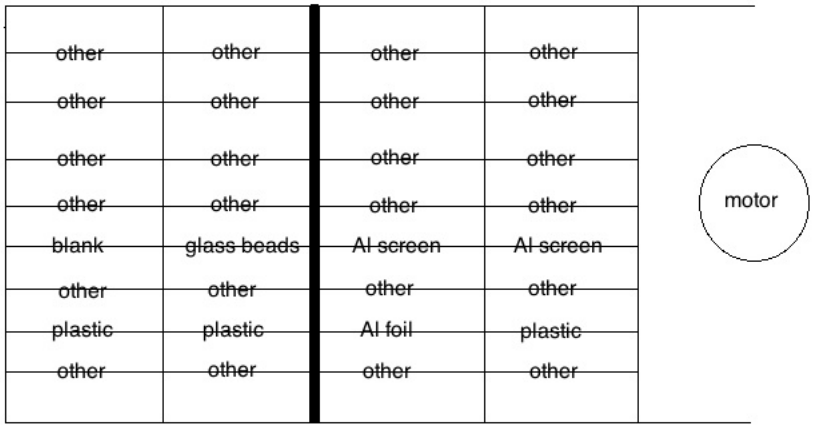
The most common diatom found in Lake Matoaka, particularly on the substrates for this study is *Fragilaria sp.*. This genus of diatom is common all over the world and has a rectangular cell wall. Renaud, et al. studied in 1999 the chemical composition of *Fragilaria pinnata* among other species of Australian algae. *Fragilaria pinnata* is a pelagic diatom which divides about 0.55 times per day, or once every 1.8 days. Its chemical content is: 34.6 percent ash, 5.8 percent carbohydrates, 14.9 percent lipids, and 25.5 percent protein. It also has the highest amount of arachidonic acid, although not lipid content, of the species studied.

3 Materials and Methods

For the first part of this study, four different substrates were used: Husky polyethylene plastic sheeting 6 mil, aluminum foil, aluminum window screening, and JewelCraft size 5/0 glass E-beads. Each substrate was set into a window frame with outer dimensions 115 cm by 87 cm. The inner dimensions were 108 cm by 80 cm, providing a surface area of 17,280 sq cm total for all substrates except the glass beads. Each of the substrates was set into the frame with a 0.175 inch spline except for the aluminum screening, which was set into the frame with a 0.140 inch spline. The plastic sheeting and aluminum window screening were splined in as is. The aluminum foil was wrapped around an already-made screen with polycarbonate screening inside. This was secured with Gorilla Tape. The glass beads were strung onto fishing line about 80 cm and tied off with approximately 5 cm tails on each side. The glass beads were each 4 mm in diameter, so the surface area total for each glass beads string was about 100 sq cm and the total for the entire frame was about 2500 sq cm. Each string was placed 4 cm apart from each other on the frame and splined in. The excess was cut off with an x-acto knife. These frames were then taken to the dock to be installed. The dock is by Keck Lab

in the middle of Lake Matoaka. There are eight slots in each of eight frames, although only the four frames closest to the motor were used for this study. Two of these slots within these frames, the second and fourth from the side furthest from Keck, were used for the study. The substrate arrangement is shown in Figure 1:

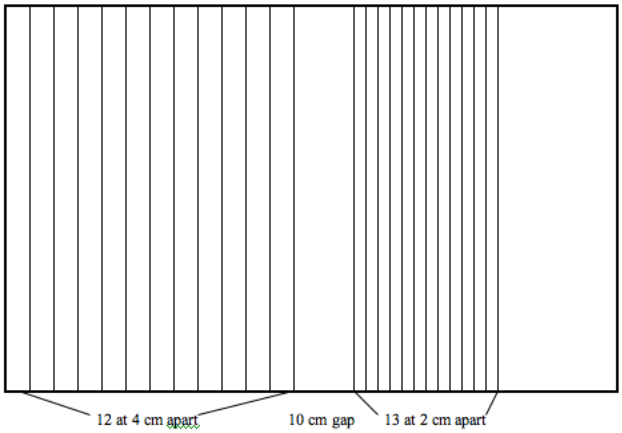
Figure 1:



The motor provided a slow but steady current through the system. The substrates in the two frames closest to the motor were put in on October 6, 2010. The plastic sheeting in the third frame from the motor was put in on October 8, 2010 and on this date the original plastic sheeting was also resplined. The glass beads and the plastic sheeting in the fourth frame from the motor were put in on October 20, 2010. The algae was monitored for growth from October 15, 2010 to November 19, 2010, generally every Friday in mid-afternoon, between noon and 4 pm. Pictures were taken of the algae on each substrate on October 20, 22, 29, November 5, 12, and 19, 2010. These pictures were then qualitatively analyzed for growth.

The second part of the experiment looked at whether the spacing of the glass beads would affect the amount of algae that was grown on them. For this, the glass beads substrate made previously was first washed with soap and water and the glass beads were re-spaced as shown in Figure 2, with a group of 13 spaced 4 cm apart and a group of 12 spaced 2 cm apart.

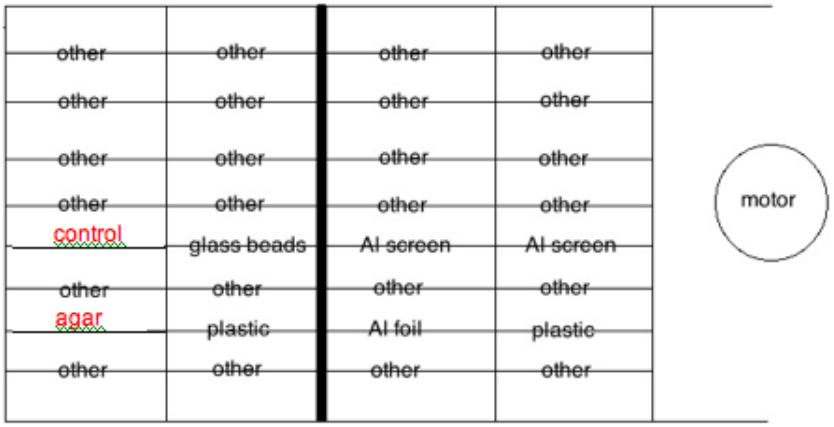
Figure 2:



The glass bead frame was put back in its original slot on February 2, 2011. The amount of algae was monitored on February 4, 9, and 16 by taking pictures, generally in the early afternoon. These pictures were then qualitatively analyzed for growth.

The third part of the experiment looked at the amount of algae that grew after priming the substrate with a layer of agar. In order to make the agar, 1.72 grams of Bacteriological Agar from SIGMA (A5306) was combined with 125 mL of water. This mixture was placed on a hot plate at about 300 ^{O} C with occasional swirling. It was kept on the hot plate until the mixture began bubbling and the agar appeared to be dissolved. The agar was then poured onto a frame with plastic sheeting, which had been prepared the same as before. The agar was smoothed with a ruler so that it would form a thin, evenly distributed film. It was allowed to sit until it had mostly solidified, for about 45 minutes, then the same process was repeated on the other side. The agar-covered plastic sheeting substrate and another control plastic sheeting substrate with no agar were placed into the dock on February 25, 2011. They were placed in the fourth row from the motor, as shown in Figure 3. The plastic sheet which was already there from the first experiment was moved down to another open row. The algae was monitored on March 2, 14, and 18 by taking pictures, generally at about 10 am. These pictures were then qualitatively analyzed for growth.

Figure 3:



This third experiment seemed to show that agar on the plastic sheeting did facilitate extra growth. So, a smaller experiment was done to determine whether the agar was in fact still there after a week and whether it was providing a greater amount of texture to that substrate. To do this, a smaller version of the substrates made in the third experiment was constructed using a piece of the aluminum window screening used previously in the first part of the experiment, with a piece of plastic sheeting 25 cm by 30 cm attached to each side of the screening using Gorilla tape. One of the plastic sheets was covered in 25 mL of agar, while the other was left bare. This substrate was then weighted using a large washer and was attached to the side of the Matoaka flume using fishing line. Pictures of the substrate both out of and in the water are found in Figures 4 and 5.

Figure 4:

Figure 5:

After one week, this substrate was taken out of the water and photographed under the microscope at 10x magnification. Both areas of high growth and areas of low growth were analyzed to determine differences in texture in each location as well as differences between the substrates in the amount of algal growth. Lastly, to determine the growth rate over time of the algae researched in this experiment, strands were cut to a

length of 2 cm at various parts along the flume and then measured each week to determine how much they had grown. These strands were cut from the tops of the aluminum bars along the sides of the slots, so that the algae was easy to reach and it was easier to determine which algae was measured. By doing this over time, one can determine both whether the algae tends to grow a lot less once it has reached a certain length as well as characterizing the growth rate as the weather heats up. This measurement study was done from March 25 to April 15.

4 Results and Discussion

The first part of this experiment examined whether using different substrates would affect the amount or rate of algal growth on them. Throughout the course of this experiment, however, most of the substrates appeared to grow algae at about the same rate. The only difference was on the glass beads frame. When compared with the plastic sheeting frame put in at the same time, the glass beads facilitated the growth of many more strings of algae than the plastic sheeting. It was unclear, however, whether the differences were because of the different substrate, or because the glass beads did not allow for as much planar growth, so the algae could only grow in long strings. A close-up of the beads showing the algal growth taken on November 5 is shown in Figure 6. In this picture the amount of growth is harder to see since the frame is out of the water and the filament structure of the algae is no longer free-flowing.

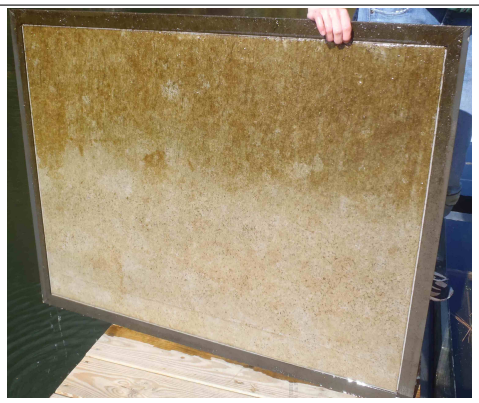
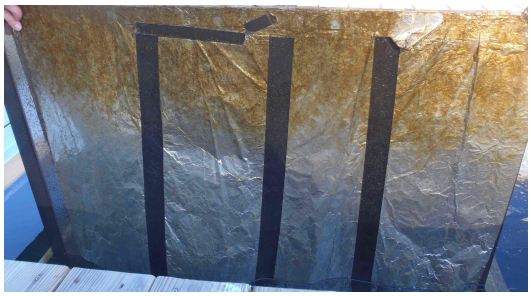
Figure 6:

The other substrates appeared to grow similar amounts of algae, although an exact quantitative measure was not undertaken. Photographs taken for the plastic sheeting, aluminum foil, and aluminum screening, all of which were put in on October 6, are shown in Figures 7-24. One representative is shown for the plastic sheeting and aluminum screening, although two substrates of each were monitored.

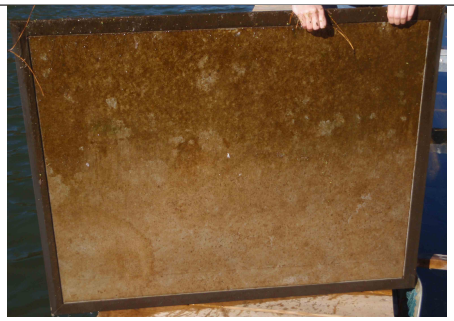
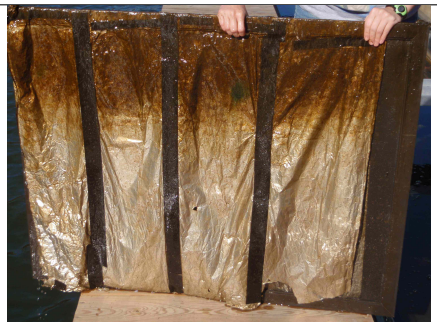
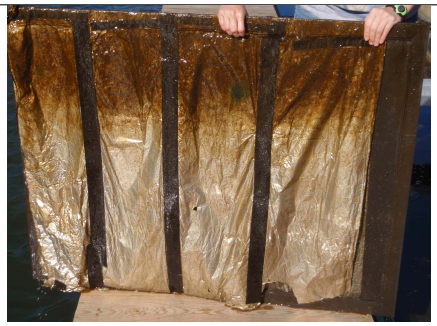
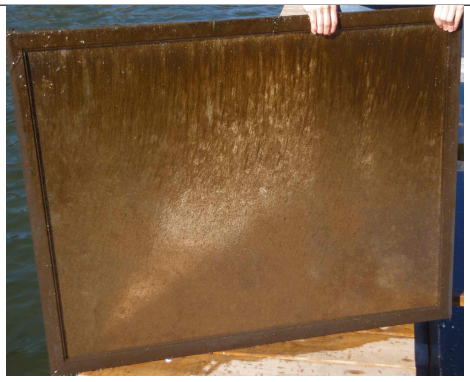
Figures 7-12

Substrate Type	October 20	October 22
Plastic Sheeting	Figure 7	Figure 10
		
Aluminum Foil	Figure 8	Figure 11
		
Aluminum Screening	Figure 9	Figure 12
		

Figures 13-18

Substrate Type	October 29	November 5
Plastic Sheeting	Figure 13	Figure 16
		
Aluminum Foil	Figure 14	Figure 17
		
Aluminum Screening	Figure 15	Figure 18
		

Figures 19-24

Substrate Type	November 12	November 19
Plastic Sheeting	Figure 19	Figure 22
		
Aluminum Foil	Figure 20	Figure 23
		
Aluminum Screening	Figure 21	Figure 24
		

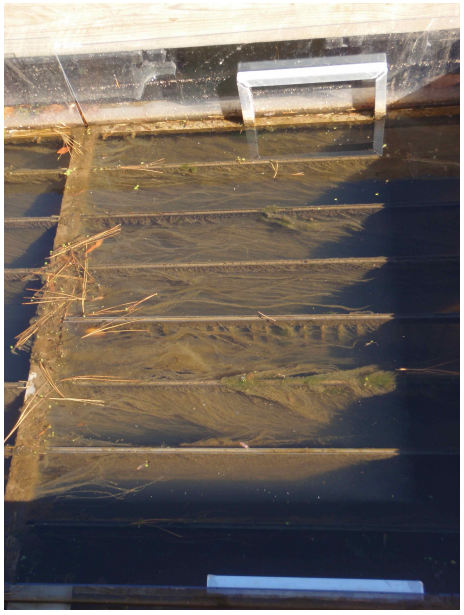
The photographs taken for the glass beads and plastic sheeting put in on October 20 are shown in Figures 25-32:

Figures 25-32

Date Taken	Glass Beads	Plastic Sheeting
October 29	Figure 25	Figure 26
		
November 5	Figure 27	Figure 28
		
November 12	Figure 29	Figure 30
		
November 19	Figure 31	Figure 32
		

There was significantly more growth in the entire chamber after November 5th, and it was unclear exactly why this occurred. There may have been more oxygen in the water or minerals. The temperature was not particularly different from the rest of the season. A picture of this, taken on November 12, is shown in Figure 33.

Figure 33:



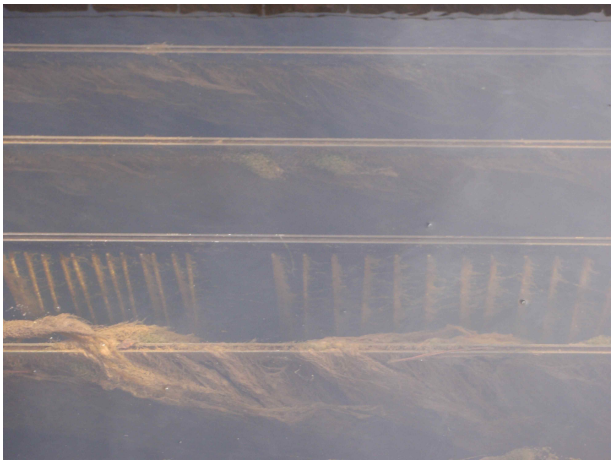
Unfortunately, almost all of the algae fell off each substrate while it was being lifted. We were unsure as to why this would occur, but it could be because there is not a very strong current in Lake Matoaka, so the diatoms do not have a need to adhere more strongly to any substrate.

There were many sources of error for this part of the experiment. Since this was done in the natural environment of Lake Matoaka, there could have been differences in nutrient content between the frames or differences in current. Also, the actual amount of algae could not be determined, and there is often bias in any qualitative study. Lastly, much of the algae sloughed off when being taken in and out of the water, so there was probably a sizable difference in the amount of algae seen and the amount of algae lost in this process. Overall, however, the project appeared to show the general progression of algal attachment over time as well as the differences and similarities between attachment to different substrates.

The second part of this experiment tried to determine whether the spacing of the strings of beads on

the glass beads frame affected the number of algae that grew on each string. This would possibly provide an insight as to whether the glass beads grew more algae because of the actual properties of the glass or because of the nature of the substrate spacing. There was much more growth over time on the strands placed 4 cm apart from each other than those placed 2 cm apart from each other. The strings with the most growth were those on the ends, to the right of the large gaps. This is shown very clearly when the frame was photographed in the water, since the strands of algae were more visible. Figure 34 shows one such photograph, taken on February 16.

Figure 34:



This seems to show that the farther apart the strings were, the more growth there was. Therefore, the fact that the strings were spread out may have contributed to the larger amount of algal growth, rather than the fact that the beads were made of glass. This may be due to a "shadowing" effect, so the algae strings brushing against each other would not allow as much growth as when strands are farther apart. The photographs for the glass beads from February 4, 9, and 16 are shown in Figures 35-37:

Figures 35-37

Date Taken	Glass Beads
February 4	Figure 35
	
February 9	Figure 36
	
February 16	Figure 37
	

There were a few sources of error with this part of the experiment as well. One would be that there was only one frame used, and that there were only spacing two spacing sizes used, 2 cm and 4 cm. Using more spacing sizes could indicate which spacing would be best for algal growth. Also, since the amount of algae was qualitatively determined by photography, rather than quantitatively measured, there may be some inherent bias. Lastly, the amount of algae was particularly difficult to characterize when the substrate was out of the water, since the strings of algae would lie flat on the beads, rather than fanning out and giving a much better indication individual string length and algae number per string. Overall, however, this seemed to show that the further apart the beads were, the more algae grew. This may imply that the stranded nature of the glass beads substrate may have given rise to the increase in algae on the beads, rather than the actual glass itself.

The third part of the experiment examined whether adding a biologic layer to the substrate would affect the amount of binding to that substrate. To determine this, agar was spread on both sides of a plastic sheet and another bare plastic sheet was used as a control. This would test the idea put forth by Bobbie, et al. (1978) that biodegradable materials may allow for more algal growth than non-biodegradable materials. Although it was more difficult to see this towards the beginning of the experiment, the agar-primed substrate appeared to have more algal growth than the non-primed substrate, particularly at the tops of the substrates. There were some sections of the agar-primed substrate, however, which had virtually no growth, while the non-primed substrate had a more even amount of algae across most of the substrate. These areas may have had a thicker layer of agar, since although the agar was smoothed, it was difficult to make it a completely even layer since it is a gel. There were some thicker agar blobs towards the top of the substrate initially, so these were most likely the areas which could have sloughed off over time or after a large rain, carrying the extra algae with it. These were only a few areas towards the top of the frame and did not show a general patchiness throughout the substrate. An arrow in Figure 42 shows one of these spots. A greater overall amount of algae, however, could be seen at the top of the substrate around those empty spots.

It is unclear exactly why the agar-primed substrate grew more algae than the non-primed substrate. This could be due to trace minerals, such as phosphates, in the agar, which the algae could use when it bound. It could also serve as a more textured surface, so that the diatoms could get a better grip on the substrate. Lastly, it could give a biologic substance to attach to, such as why algae can sometimes bind to other algae more easily than starting a whole new strand of algae on a bare substrate. More experiments would need to be done to determine exactly which of these hypotheses can be substantiated, or if a combination is true. The photographs of the agar-primed and non-primed substrates taken on March 2, 14, and 18 are shown in

Figures 38-43:

Figures 38-43

Date Taken	Agar-Primed Sheet	Non-Primed Sheet
March 2	Figure 38	Figure 39
		
March 14	Figure 40	Figure 41
		
March 18	Figure 42	Figure 43
		

There were many sources of error this part of the experiment. There was only one set of frames, so there was no check in the amount of variation between different agar-primed substrates or non-primed substrates. The amount of agar was also not completely even across the agar-primed substrate, which did not allow for a standard thickness for comparison, and may have led to the large holes in the algal growth seen, as discussed earlier. Lastly, the difference in the amount of algae on each substrate was qualitatively, rather than quantitatively characterized, which leads to a possibility of more bias. The data does seem to show, however, that there are differences in algal growth depending on whether the substrate was primed with agar or not.

After finding that agar may help to increase algal growth, a smaller experiment was undertaken to see why this may be occurring. After being put into Lake Matoaka for one week, the smaller substrate, including both an agar-primed plastic sheet and a bare plastic sheet, was analyzed under a microscope. Both sheets seemed to have about the same amount of algal growth on them, except for one larger pocket of algae on the agar-primed substrate. This was not completely counter to the previous experiment, since there was less noticeable difference in the growth at the one week mark between the agar primed and non-agar primed substrates analyzed earlier. There were still noticeable clumps of agar on the agar-primed substrate after one week, just as was seen in the previous experiment. These clumps, however, were not very textured and more just gentle rises and falls. Most of the small bubbles seen on the plastic were actually water droplets, rather than actual bumps in the substrate. The places with the most amounts of agar did not correlate with the places which were bumpiest. These pictures were all taken at 10x magnification, and an extra picture was taken at 50x of the agar-primed substrate to determine if there was more texture that could be seen at a higher magnification. There did not appear to be any more roughness exposed at the higher magnification. At this magnification, the best resolution that one could probably make out was about 10 micrometers.Each of the three pictures taken for each magnification, including the picture of the large growth of algae from the agar-primed substrate, are shown in Figures 44-49, in addition to the 50x magnification picture, shown in Figure 50. Each of the bars on the bottom right represents 1 mm in that figure.

Figures 44-50

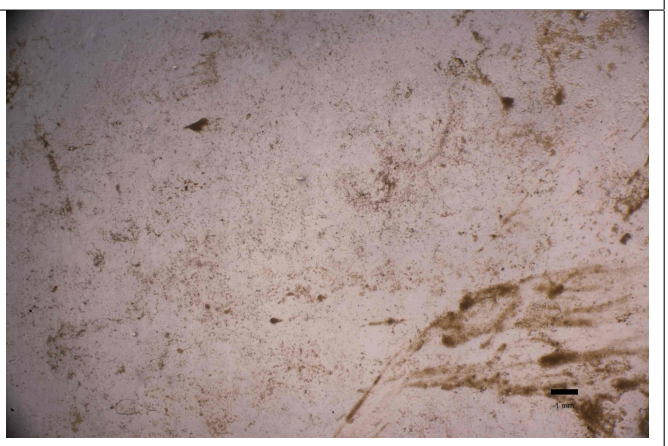
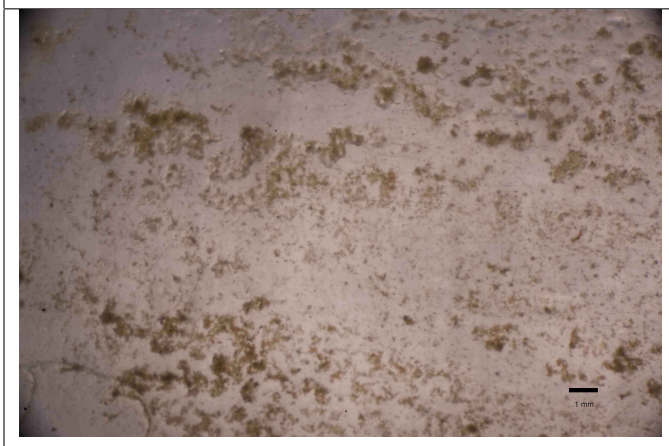
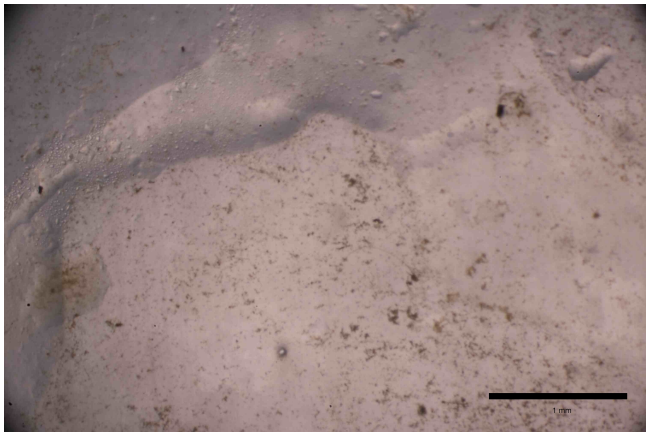
Agar-Primed Sheet	Non-Primed Sheet
Figure 44	Figure 47
	
Figure 45	Figure 48
	
Figure 46	Figure 49
	

Figure 50:



There were many sources of error for this part of the experiment as well. Since this was only done with one substrate and over the course of one week, there was nothing to compare this substrate to besides the larger substrates used earlier. If the experiment had been done for longer as well, we may have been able to better track the growth of algae over time better through microscopy, although the process of putting the substrate underneath the microscope did include taking each part apart, making the substrate difficult to put back into Lake Matoaka. This part, however, did seem to show that texture may not be the reason for the increased algal growth on the agar substrate and may point towards the biologic nature of the agar itself being the reason.

The last part of this experiment was to analyze growth rate over time. Unfortunately, this was not done over a long period. Our cutting and measuring technique did give some crude measurements for doubling time. All strands, when cut, were two centimeters in length. There was a smaller amount of growth in the first week, measured on April 1, with a doubling time of about 5-6 days, but the second week, measured on April 8, showed much larger cumulative growth. This was evident throughout Lake Matoaka, with huge blooms of algae outside the flume as well. The third week had a middle amount of growth of about 3-5 days. The doubling times and growth measurements are given in Table 1. The measurements given are for clumps of algae, since there was generally a large group of strings, rather than one string by itself; values are given as a range and not a single value. There were insufficient measurements to compute a mean and standard deviation.

Table 1

	March 25	April 1	April 8
April 1 Measurement	4-5 cm	2 cm	
April 1 Doubling Time	5-6 days		
April 8 Measurement	6.5, 84, 107 cm	5,12,17	2 cm
April 8 Doubling Time	1.5-1.75 days	2.2-2.6 days	
April 15 Measurement		25, 30, 35 cm	9, 10-15 cm
April 15 Doubling Time		4.5-6.5 days	2.5-3 days

Since there were so few days on which these measurements were taken, there is a wide variation. Because of this, more weeks and more cuttings would need to be done to determine how much the length of the algae string or the weather would have an effect on the growth rate. The growth rate, however, does seem to be between 1.5 and 6.5 days, regardless of the week and age of the algae, although the large bloom of algae did seem to have an effect on this growth value as well. This is a bit larger than as the doubling rate that Renaud, et al (1999) found of 1.8 days. These differences in growth may be due to weather changes rather than length of the algal string, since some, such as those cut on March 25, appeared to grow much more as it was longer while others, such as those cut on April 1, grew much less. From all of the experiments, it seems to show that there are some substrate characteristics, such as linear growth, spacing distance, and agar priming that make a difference in algal growth. The glass beads supported linear growth and spacing between strings. Plastic sheets and metal substrates supported planar growth and agar priming. The exact causes between different growth rates are still unclear and no single substrate provided all the advantages.

5 Conclusion

The purpose of this experiment was to determine the effect of various substrate properties on the amount of diatoms attached. Three major properties were examined: the substrate material itself, the compactness of the substrate material, and priming the substrate with a layer of agar. For the first part of the experiment, four substrates were used: plastic sheeting, aluminum foil, aluminum screening, and glass beads. There was no significant difference between the amounts of algae found on the substrates except for the glass beads, which showed a higher growth of algal strings, particularly towards the beginning of the experiment. This should be useful for determining the best substrate to grow the algae on for future harvesting, both in

Lake Matoaka and in the York River. The second part of the experiment examined whether spacing out or compressing the strings of glass beads would affect the amount of growth on each strand. The strands were either placed 4 cm apart or 2 cm apart. The strands which were 4 cm apart showed much more growth than those 2 cm apart, and the strands with the most growth were those on the end of each group, next to a large gap. This seems to indicate that the difference in algal growth between the glass beads and the other substrates may be due to the linear nature of the substrate rather than the glass itself. This could indicate that a linear substrate is best, and therefore strings of rope or beads provides an advantage over full screens for growing more algae. The third part used an agar-primed plastic sheet and a non-primed sheet to determine whether adding a biological layer to the substrate would affect attachment of diatoms. There did appear to be more growth on the agar-primed substrate than the control; although further experiments seemed to show this is not the result of agar texturizing the substrate. This information could be used for future harvesting, since covering the substrates in a layer of agar or another biologic substance could allow for more growth than just leaving the substrates bare. The growth rate determined for this algae does also indicate that the algae in Lake Matoaka grows at or a little slower than the rate expected.

These results give many ideas for other avenues of research in the future. The optimal placement of a linear substrate, such as rope or glass beads as well as bead size could be informative. A linear substrate could be set up and used for future harvests that maximized the amount of algae growing, perhaps even by rotating the substrates 90^O so that there would be more space between substrates. Other substrates, such as other linear substrates, could also be tested to see whether there would be a sizable difference in algal growth. Specific properties of the agar, such as the amount of trace elements in it, or the biologic nature of it, could be tested to see whether any of these could be the reason why the algae seems to bind to it more readily than to just the bare substrate. Other areas, such as the York River site, could also be tested to see if these results are also demonstrated in a different location, or whether variables such as current have a stronger affect on the outcome. These results, however, are promising for indicating various ways to improve the quantity of harvestable algae from substrates based on the substrates' properties supporting diatom attachment and growth.

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