Analyzing the Effects of Surface Energy on Algae with Specialized Attachment Mechanisms

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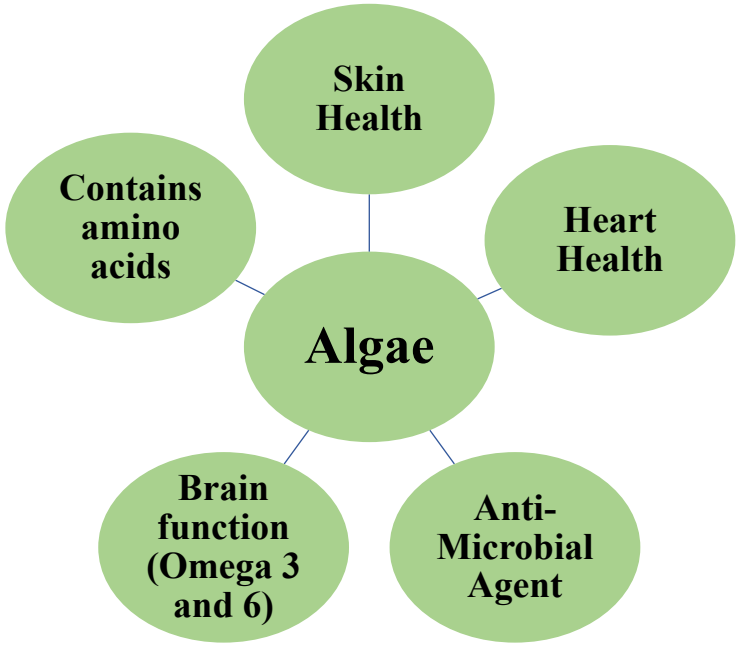
The Importance of Algae

- Algae potentials: Wastewater treatment, catching runoff fertilizer, animal feed, nutritional supplements, biofuel, and more
- Algae has recently been popularized by media due to its potential as a biofuel and ExxonMobil's commercials on their algal research

https://geneticliteracyproject.org/2018/01/15/new-generation-gmo-crops-dramatically-boost-biofuel-production/



https://www.mnn.com/earth-matters/animals/stories/20-things-you-didnt-know-about-cows

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The Importance of Algae

- One of the complications that come with algae are the inefficiency and cost of the harvesting process
- Most systems are designed for suspended growth of microalgae
- Research has been focused on microalgae rather than filamentous algae

Credit: Pacific Northwest National Laboratory

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The Importance of Algae

- Filamentous algae preferable because they grow attached, can be mechanically harvested
- Much of filamentous algae's early stages of attachment are unknown
- Knowledge of attachment behavior can allow for manipulation of substrates to encourage or discourage growth

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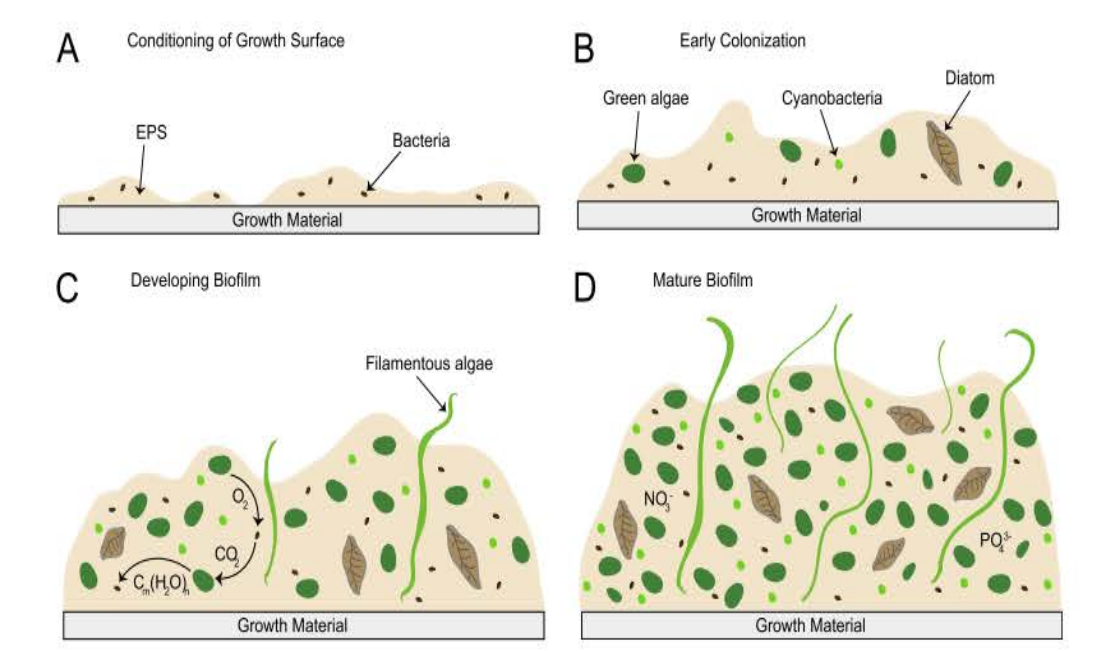
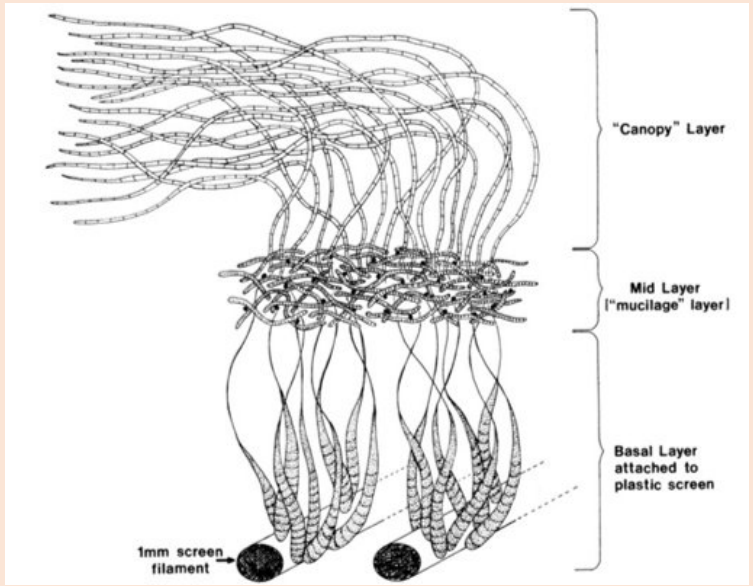
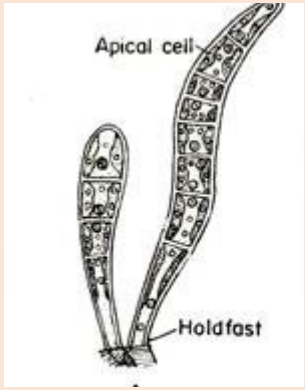
Micro Algae vs Filamentous (Macro) Algae

Micro algae attach to a surface that has been conditioned by bacteria and extracellular polymeric substance (EPS) and as the algae attach they form a biofilm on the surface



Limitations arise when the biofilm grows larger, it becomes difficult for algae near the growth material to receive light and nutrients





Grows more like a "normal plant" so the limitations that exist for microalgae do not apply

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Current Work

Filamentous algae as found naturally in rivers and creeks:

https://kymkemp.com/2017/07/21/officials-issue-cyanobacteria-blue-green-algae-warning/
https://www.landcareresearch.co.nz/resources/identification/algae/identification-guide/identify/guide/filamentous/microscopic/unbranched/chloroplasts-green-yellow/wall-featureless/rhizoclonium

- Previous research covered microalgae growth and attachment (*S. dimorphus*)
  - Developed protocol for microalgae culture growth, measurement, and attachment
- Filamentous algae respond best to flow environments
- Needed new protocol catered to filamentous macroalgae

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Current Work- Cultivating

Tribonema and Stigeoclonium cultures with bubbling

- Filamentous algae used: Oedogonium, Tribonema, and Stigeoclonium
- Pure algae strains from University of Texas
- Started growth in vial, fed Bold 3N Medium
- Kept under a growth lamp 24/7

Oedogonium in a vial, very low concentration

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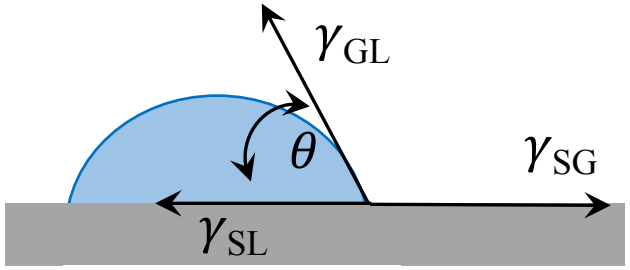
Calculating Surface Energy

- The van Oss contact angle method was used to obtain surface energy parameter values to fit in the modified Young's Equation below
- Used 3 probe fluids: hexadecane, ethylene glycol, water



γ_{SG}= Solid-Gas
γ_{SL}= Solid-Liquid
γ_{GL}= Gas-Liquid

Modified Young's Equation

$$cos\theta = -1 + \frac{2(\gamma_{sr}^{LW}\gamma_{l}^{LW})^{1/2}}{\gamma_l} + \frac{2(\gamma_{sr}^{+}\gamma_{l}^{-})^{1/2}}{\gamma_l} + \frac{2(\gamma_{sr}^{-}\gamma_{l}^{+})^{1/2}}{\gamma_l}$$

This term accounts for Lifshitz van der Waals forces

These two terms account for acid-base pair/ polar interactions

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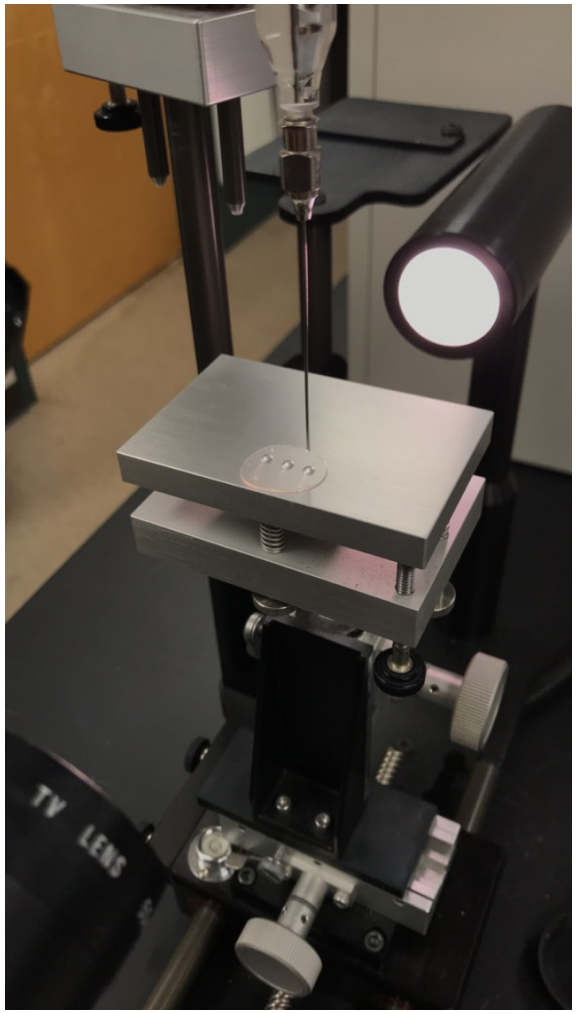
Calculating Surface Energy

Probe Fluid	Average Contact Angle
Water	73.0°
Ethylene glycol	48.9°
Hexadecane	17.2°



Surface Energy Parameters (mJ/m^2)		
γ^{LW}	γ^-	γ^+
26.3	11.5	1.3

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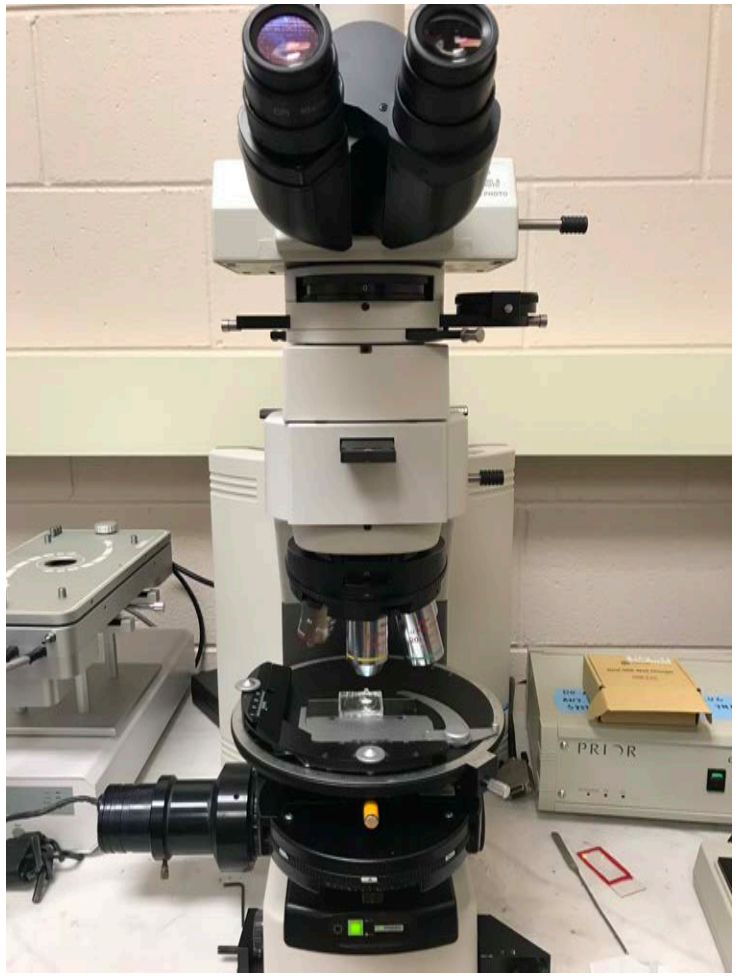
Filamentous Algal Attachment Tests

- Substrates tested: glass, polylactic acid (PLA) disks
- PLA disks made via tortilla press
- Substrates were placed in glass containers filled with 20 mL ultra-pure water and 10 mL of undiluted algae, then left undisturbed for 24 and 48 hrs
- Distortion of the images occurred due to water film



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Filamentous Algal Attachment Tests

This “star” formation is the specialized attachment mechanism for Stigeoclonium

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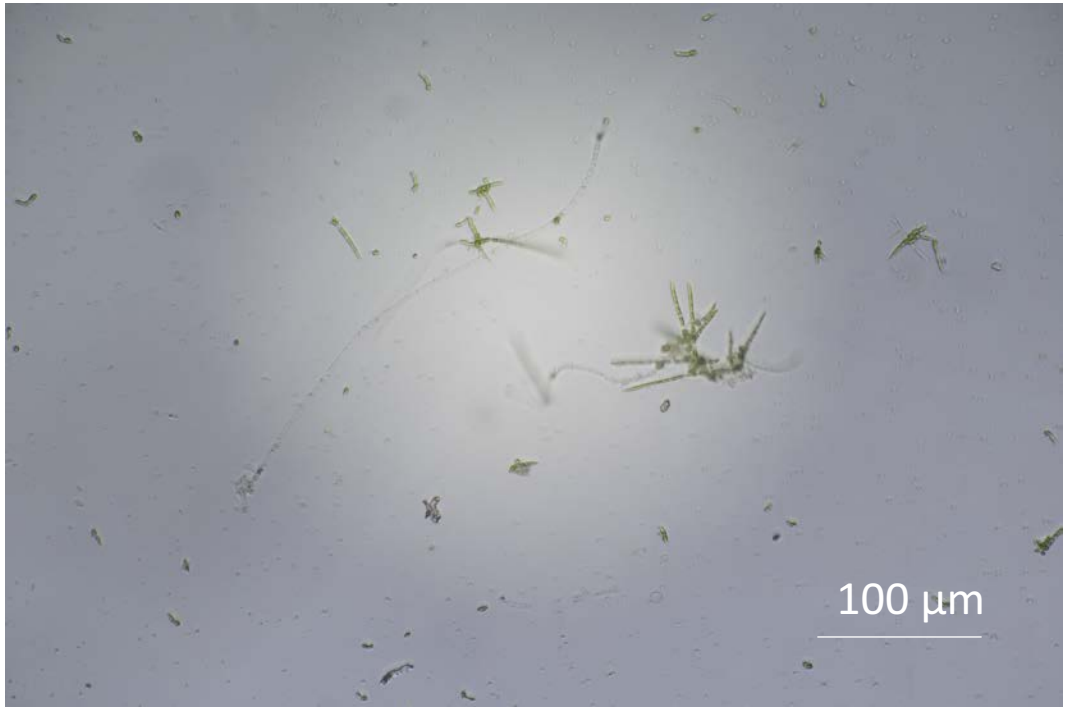
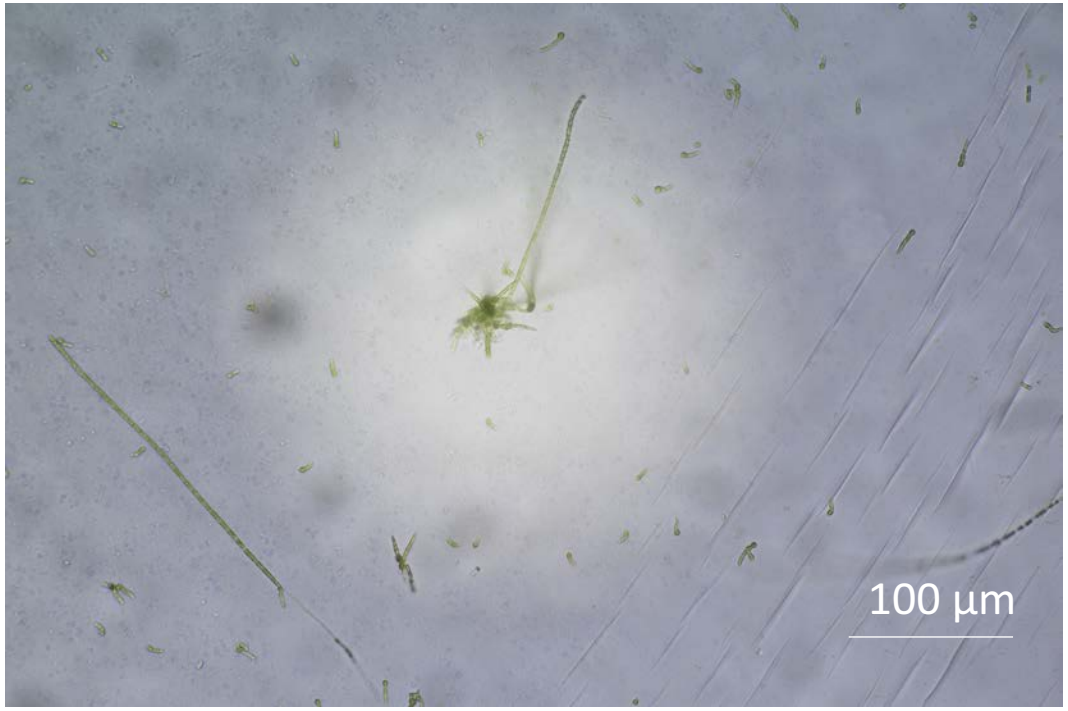
Filamentous Algal Attachment Tests

Stigeoclonium on Glass

48 hrs



Stigeoclonium on PLA



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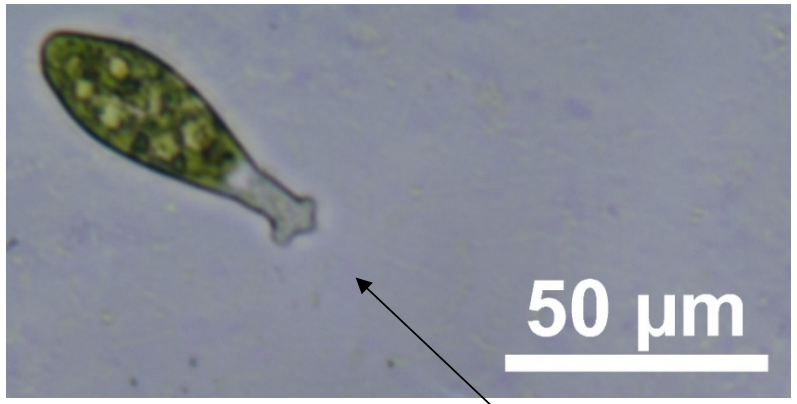
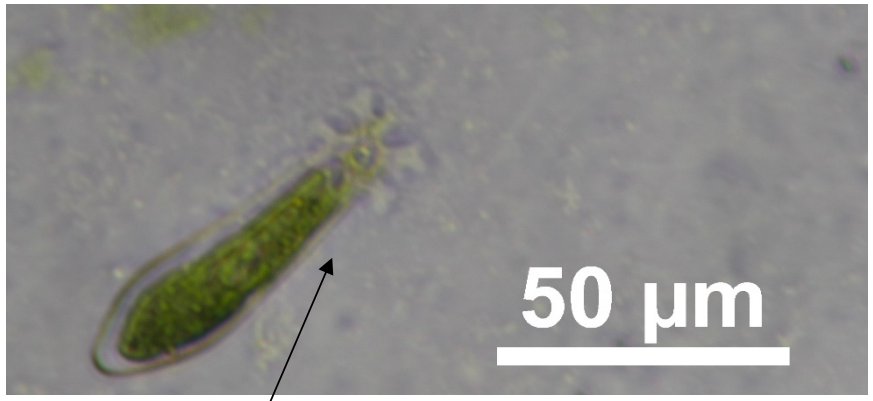
Filamentous Algal Attachment Tests





Oedogonium specialized attachment mechanisms are holdfasts, small “hand”

Pictures taken by Graduate Student Zahra Karimi for her work

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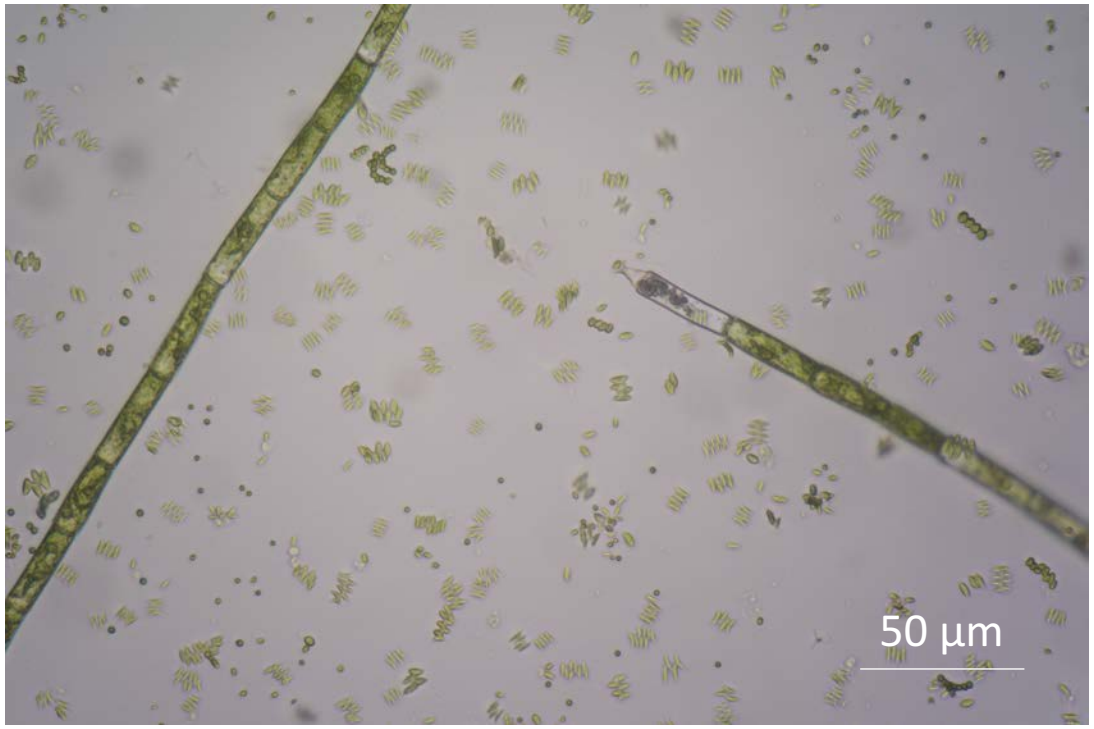
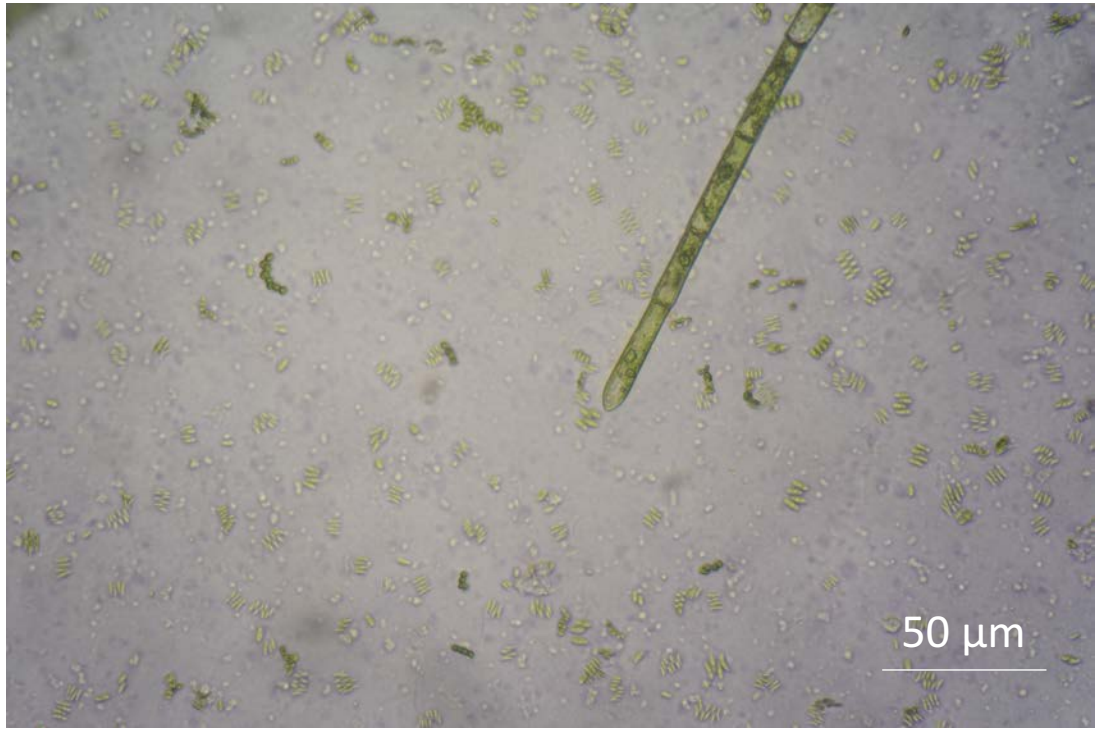
Filamentous Algal Attachment Tests

Oedogonium on Glass 48 hrs **Oedogonium on PLA**





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Conclusions

- Filamentous macroalgae are more difficult to grow in a lab environment, a protocol for keeping a culture was made
- Successful attachment with Stigeoclonium to both glass and PLA
- No successful attachment yet with Oedogonium, likely due to filamentous strand length

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Future Work

- Additional substrates, such as Teflon, for attachment tests
- Attachment tests varying light exposure
- Cutting Oedogonium filaments to see the affect on attachment

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Acknowledgements

- The Virginia Davis Research Group
- The Undergraduate Research Fellowship Program of Auburn University

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Questions?

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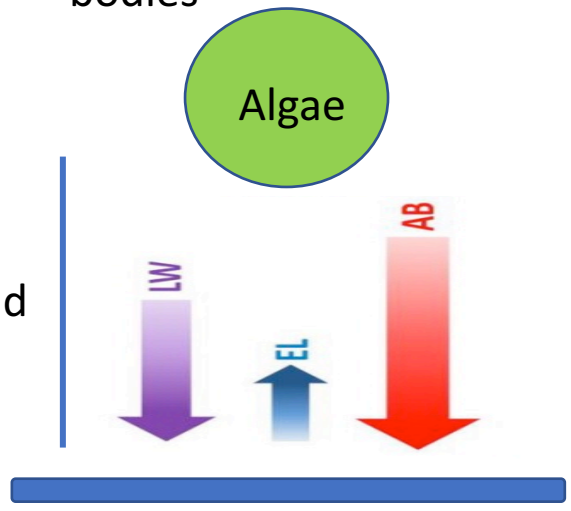
Calculating Surface Energy

Modified Young's Equation

$$cos\theta = -1 + \frac{2(\gamma_{sr}^{LW}\gamma_l^{LW})^{1/2}}{\gamma_l} + \frac{2(\gamma_{sr}^{+}\gamma_l^{-})^{1/2}}{\gamma_l} + \frac{2(\gamma_{sr}^{-}\gamma_l^{+})^{1/2}}{\gamma_l}$$

- Accounts for Lifshitz van der Waals forces
- VdW: dipole-dipole (polar), dispersion (nonpolar), and hydrogen bonding
- Lifshitz: no pairwise additivity, looks at bulk whole bodies

- Accounts for acid-base/polar interactions
- Attraction or repulsion based on polarity



$$G^{TOT}(d) = G^{AB}(d) + G^{LW}(d) + G^{EL}(d)$$

G^{AB} Acid-base Interactions

G^{LW} Lifshitz-van der Waals Interactions

G^{EL} Electrostatic Interactions

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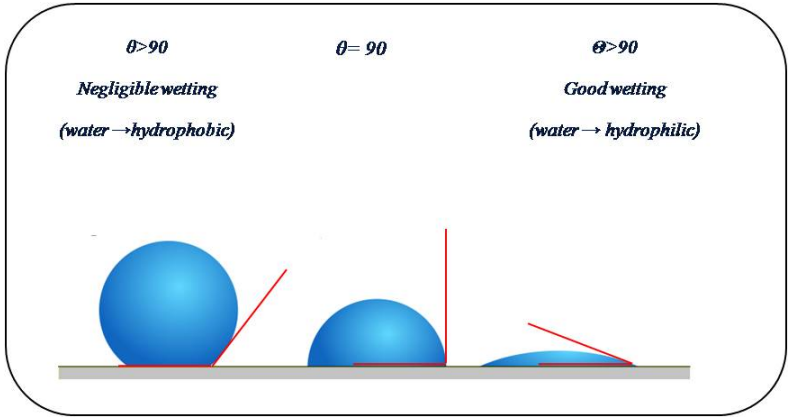
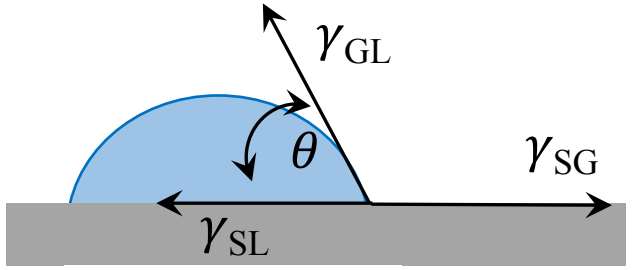
Calculating Surface Energy





γ_{SG}= Solid-Gas
γ_{SL}= Solid-Liquid
γ_{GL}= Gas-Liquid

Three Liquids used:
Acid/base value of zero: Hexadecane
Polar: Water
Nonpolar: Ethylene Glycol

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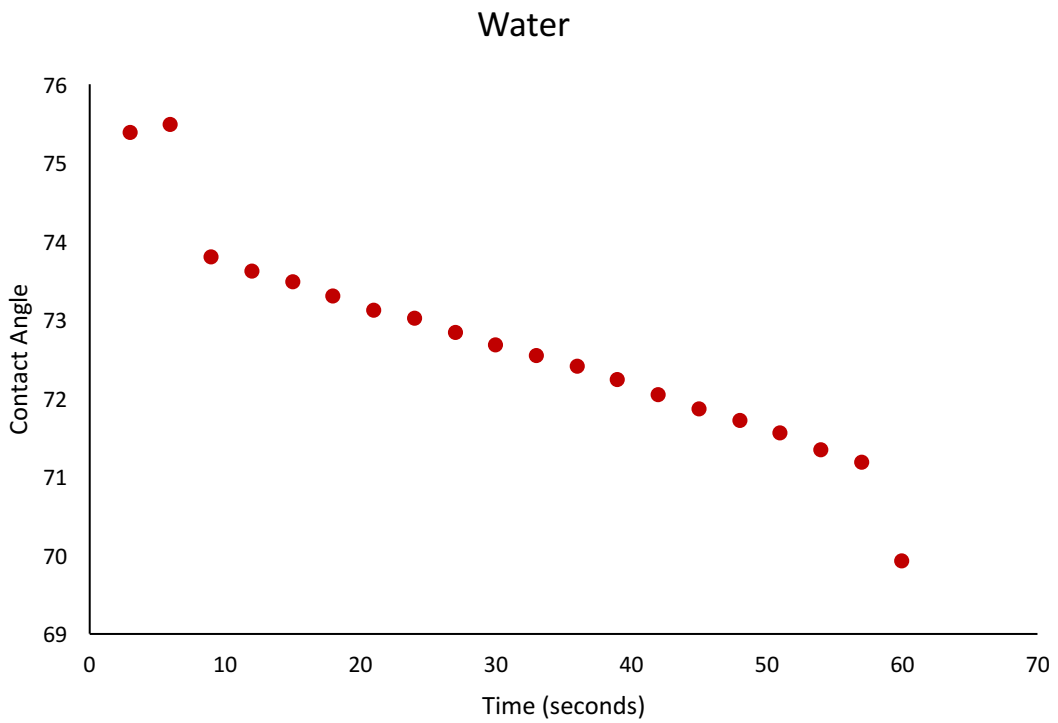
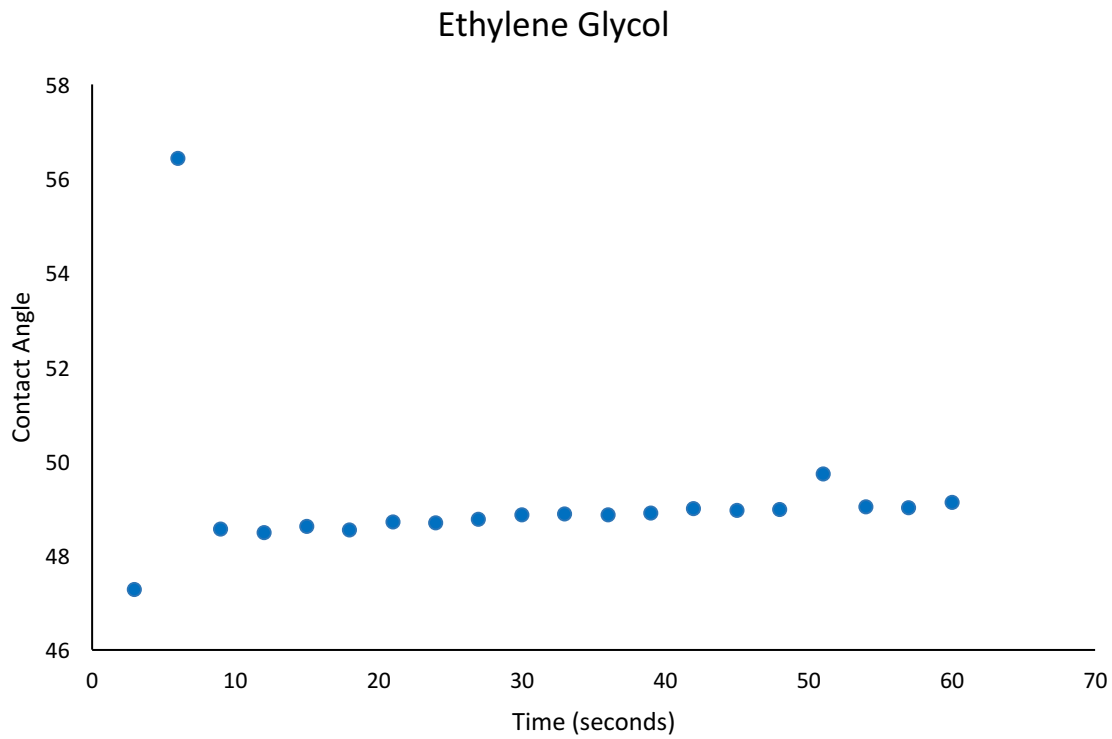
Calculating Surface Energy





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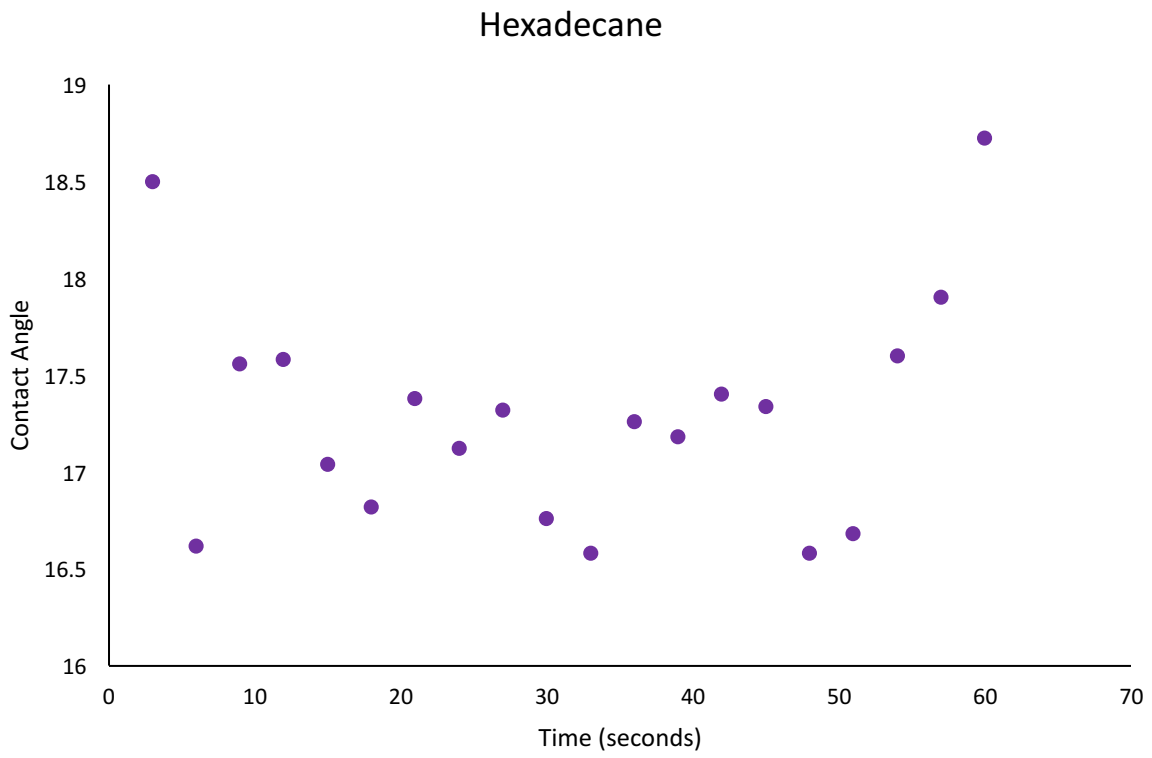
Calculating Surface Energy



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Bold 3N Medium

#	Component	Amount	Stock Solution Concentration	Final Concentration
1	$NaNO_3$ (Fisher BP360-500)	30 mL/L	10 g/400mL dH_2O	8.82 mM
2	$CaCl_2 \cdot 2H_2O$ (Sigma C-3881)	10 mL/L	1 g/400mL dH_2O	0.17 mM
3	$MgSO_4 \cdot 7H_2O$ (Sigma 230391)	10 mL/L	3 g/400mL dH_2O	0.3 mM
4	K_2HPO_4 (Sigma P 3786)	10 mL/L	3 g/400mL dH_2O	0.43 mM
5	KH_2PO_4 (Sigma P 0662)	10 mL/L	7 g/400mL dH_2O	1.29 mM
6	NaCl (Fisher S271-500)	10 mL/L	1 g/400mL dH_2O	0.43 mM
7	**P-IV Metal Solution**	6 mL/L		
9	**Soilwater: GR+ Medium**	40 mL/L		
10	**Vitamin B_{12} Solution**	1 mL/L		

https://utex.org/products/bold-3n-medium