SEAGRANT

Optimal Temperature Range for Maximum Lipid and Pigment Yields from Local Louisiana Algae Strains

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Tel M Rouse

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Research Advisors:

Dr. Kelly A. Rusch

Dr. Teresa Gutierrez-Wing

Global Energy Crisis

In the past several decades, developed nations have put forth great amounts of R&D into energy generation and conservation (Chisti, 2007). With the amount of time and resources that modern society has put into the discovery and implementation of new energy technologies, it is obvious that there exists a great demand for alternatives to the current fossil fuel products that dominate the market today. Throughout modern society's experience with the luxuries of modern transportation, industry, and electric appliances, there has existed many environmental, political, moral, and economic reasons for guiding changes within each developed nation's energy market. For example, the oil embargos and shortages of the 70's spurred massive alternative energy research in America, which felt that energy dependency on any level could lead to national security issues (Chisti, 2007). Today, the driving force in America's pursuit of alternative energy made apparent by the federal government's regulations and policies is environmental concern. Though strict policies have been enacted and enforced on industries, major implementation of new technologies has persistently evaded both America and many other nations around the globe.

A great resource to study modern successes and failures of alternative energies is European politics and energy policies (MacKay, 2008). Europe, with a dense population and scarce fossil fuel resources, has much more incentive to implement alternative forms of energy than the United States. In locations with few available natural resources and tough competition to acquire energy to meet national demands, it is quite evident how immensely political and emotional energy policies can become in the European theatre. In many European countries, such as France, the UK, and Germany, there has existed a great a political divide between renewable and nuclear energy for replacing fossil fuels. In the early 2000's, nuclear power was clearly receiving much more support, as many in the scientific community scoffed at renewable energy as a viable alternative to nuclear energy. However, it is clear that in recent years, especially after the Fukushima Daiichi Nuclear Power Plant incident, that many countries are taking a hard stance against nuclear power, with the most extreme example being Germany, which plans to phase out all nuclear power by 2022 (Dempsey, 2011). Though this one instance does not signify the end of nuclear power, it does signify that there currently exists a real demand for renewable energies in the near future (Nigam).

The Future of Renewables in America

It is evident that there is no final energy solution in near sight for any society. Until a superior technology emerges, continued research and implementation of various renewable technologies will endure. As evident with global trends, these new technologies will see continuing increases in implementation as old technologies become outlawed or more expensive to maintain due to increasing tariffs and regulations. America is caught in an especially precarious political position, because it is fortunate enough to possess a low population density, vast amounts of hydrocarbon deposits, and vast amounts of capital suitable for the implementation of various renewable energies. Though America should loosen its dependence on fossil fuels and ultimately will, it is evident that America's transition will be much more gradual than that of European countries and will consist of a wide variety of improved non-renewable and renewable resources. Each region will have its own economic or political reasons to utilize particular renewable technologies along with current gas, coal, and fuel production methods that meet the new environmental standards. For example, the deserts of southwest America are an excellent location to harness solar energy, (Leone, 2011) the vast central plains provide enough wind for feasible investment in wind energy, and the expansive coastline will ultimately be lined with offshore wind farms and tidal energy farms. However, as technologies currently stand, each one of these alternative renewables are fraught with technological and economic hurdles that must be overcome with continued research (MacKay, 2008).

Going Green

Numerous alternative energies have been proposed within the last four decades, and each has its own strengths according to geography and economic sector. However, the process of scaling up alternative energy production to match that of current energy consumption has proved to be a very difficult and lengthy process (Singh, 2010). It is for this reason that biofuel, though faced with its own technical and economic hurdles, remains a very attractive, worthwhile investment to researchers and energy companies alike. Biofuels would serve as a realistic supplement to current petroleum fuels used in automotive transportation and other machines running on combustible engines. Though it is unquestionable that electric engines are much more efficient than combustible engines and will undoubtedly dominate the future of transportation, current battery technologies and limited electrical infrastructure will prevent electric vehicles from becoming the dominant renewable energy for several years to come (MacKay, 2008).

Biodiesel from Microalgae

The most lucrative aspects of biofuels are that they can feasibly be mass produced on very large scales, are easily transportable, and easily implementable into the current energy market without any need for change in the existing energy infrastructure. (Chisti, 2007). Of all biofuels current produced, biodiesel seems to be the only viable option for large-scale production. Though many varieties of biofuels can be produced from an array of food crops and cellulosic materials, the redistribution of agricultural resources that were once reserved for food crops have shown to cause numerous adverse economic consequences in other sectors of the agriculture industry, namely food crops (Mata, 2010). Thus, many researchers and industries alike have turned their focus specifically to biodiesel production from microalgae. Microalgae prove to yield much greater lipid content per unit area of culture than other crop alternatives, yet require phenomenally less agricultural resources such as water, soil, and the necessary machinery and space for harvesting and storage (Singh, 2010). Perhaps the most important fact when concerning the future of biofuels is this: even after our standard vehicles are all converted to more efficient electric vehicles, there will remain for a long time many vehicles that will not be easily converted to electric vehicles (MacKay, 2008). Large industrial and transport vehicles, such as dump trucks, cranes, and other construction and mining vehicles that require large amounts of torque and power over extended periods of time will continue to demand the reliability of internal combustion engines. Thus, if Louisiana, one of few geographic locations with the perfect resources for low-cost biodiesel production were to develop a sufficient infrastructure, it could potentially be the leading national or even global supplier of sustainable heavy machinery fuels for decades to come. However, in order for biodiesel from microalgae to be economically feasible and competitive with hydrocarbons, production costs must be reduced and functionality of nearly every component and stage of processing must be maximized for profit (Singh, 2010). Current utilization of microalgal byproducts include food, fertilizers, colorants, dyes, feedstock, pharmaceuticals, wastewater treatment and carbon capturing (Mata, 2010).

Scaling up Biofuels Operations with Microalgae

Since algae gained attention as a possible renewable energy source in the 1970’s, much research has been done on various parameters that effect the growth and lipids of algae (Mata, 2010). Of these parameters, temperature is one of the most important variables to consider when deciding to scale up algae production for biomass feedstock, as the energy input needed to maintain optimal growing conditions for a specific strain could prove economically unfeasible. The research team under Dr. Teresa Gutierrez-Wing has isolated a Chlorella/cyanobacteria co-culture from LSU University Lakes, Baton Rouge, Louisiana, and we are currently investigating the feasibility of using this strain to produce biomass for lipid extraction under local Louisiana conditions. The co-culture produces lipids that can be used for biofuels conversion, and it also shows promising signs of resilience to local conditions. This project will attempt to mimic a range of temperatures found during Louisiana's summer months (the ideal growing season for harvesting algae). Three temperatures (25 °C, 30 °C, and 35 °C) will be simulated, and the growth, lipid production, and pigment production of the algae will be monitored to determine the effects (if any) of the outdoors seasonal environment on the co-culture strain.

Project Objectives

The project objectives are to mimic the temperature range found in the Louisiana summer growing season to determine the feasibility of scaling up algal production of the local co-culture for biodiesel production. Temperature effects on biomass density, lipid yields and concentrations, and pigment concentrations will be recorded and analyzed. Afterward, a separate experiment concerning lipid extraction temperatures will be performed to determine feasibility of extra energy input into the agitation phase could result in lipid gains from increased extraction efficiency.

Methods and Materials

Experimental Design: A total of four experiments were run throughout the duration of this project. Each experiment consisted of collecting the co-culture from an already isolated state and then acclimating it slowly to new temperatures and media conditions. Then, the co-cultures were diluted and allowed to grow, so that growth rate and biomass could be measured for analyses. Once the co-cultures exited the growth phase and transitioned to the stationary phase, the cultures were harvested. Afterward, lipid extractions were performed on the harvested samples to determine how the various temperature environments affected lipid concentrations. For the fourth and last experiment, additional lipid extractions were performed at higher temperatures (35 °C and 50 °C) to investigate the returns of additional energy input on the lipid extraction.

Experimental Setup: The experimental setup consisted of a platform with an overhead rig of 4X40 Watt fluorescent lights which provided ~120 µmol/m^2*s to the samples below. Resting on the platform were two large tubs in which the various temperature baths would incubate the samples at the appropriate experimental temperatures of 25 °C, 30 °C, or 35 °C. A single aeration pump with a bacteria filter provided a constant flow rate of 0.4 cfm to each sample. Clear airline tubing was sterilized in 10% bleach solution and rinsed well with deionized water before use.

Figure 1: Experimental setup for 25 °C conditions displayed (acclimation phase)

A clean 1000 µl pipette tip was fixed to the end of each piece of tubing inserted into the media to insure sterility. Media was prepared by autoclaving a desired amount of deionized water and then adding 0.74 ml/L F/2 media to the autoclaved water. Before the experiment began, dense cultures of algae were transferred from the basalt inoculum to F/2 media and left to adapt to the new conditions of the experimental setup over the course of at least 4-5 days or until adequate densities were reached. Once the inoculum had reached appropriate densities, the inoculum was added to the prepared F/2 media flasks in 1/5 dilution. Triplicates were run for each variation in temperature.

Experimental Procedure

Immediately after the inoculum was added to the prepared media to begin experimentation, the biomass was recorded using Total Suspended Solids (TSS) for Freshwater Algae Matrix method (Standard Methods 2450D). The initial optical density was also taken along with serial dilutions using the Hach DR 4000 Spectrophotometer at 664 nm λ, and ion chromatography was performed (Dionex DX 320 IC, Standards and Methods 4410) to determine initial Nitrate and Phosphate concentrations in the samples. The optical density was recorded each successive day and plotted in a graph. The algae would be harvested immediately after reaching the stationary phase of growth, i.e. slope equal to zero. On the day of harvesting, TSS would be performed to record a final biomass reading of the samples, and ion chromatography would be executed to determine final nitrate and phosphate concentrations. 100 ml of each sample was then collected for lipid extraction. 100 ml portions of each sample of algae were concentrated to algal mass pellets through centrifugation at 5000 RPM for 10 minutes. Afterwards, the Folch method (chloroform/methanol 2:1 at 20 minutes agitation (Folch et al., 1957)) was utilized to extract the lipids from each sample. The collected lipids from each sample were then transferred to pre-weighed 13X100 mm test tubes and dried with nitrogen. Afterward, the final weight of the test tubes was measured, and the amount of lipids was calculated in each sample per volume. Fluorescence using an S-T 450 Fluorometer (Standard Methods 10200H) was utilized for the measurement of chlorophyll a.

Figure 2: Hach DR 4000 Spectrophotometer

Figure 3: Filtration setup used in TSS

Results

Preliminary Experiments Overview: The first two experiments were utilized to practice the necessary techniques for successful growth and extraction of lipids from the co-culture. These experiments were very useful in the development of the working procedures used in the Growth and Extraction Experiments later on. Therefore, these two experiments are labeled as Preliminary Experiments 1 and 2. For both of these experiments, one batch of triplicates was grown at 25 °C while the other batch of triplicates was grown at 30 °C.

Preliminary Experiment 1 - 25 °C and 30 °C

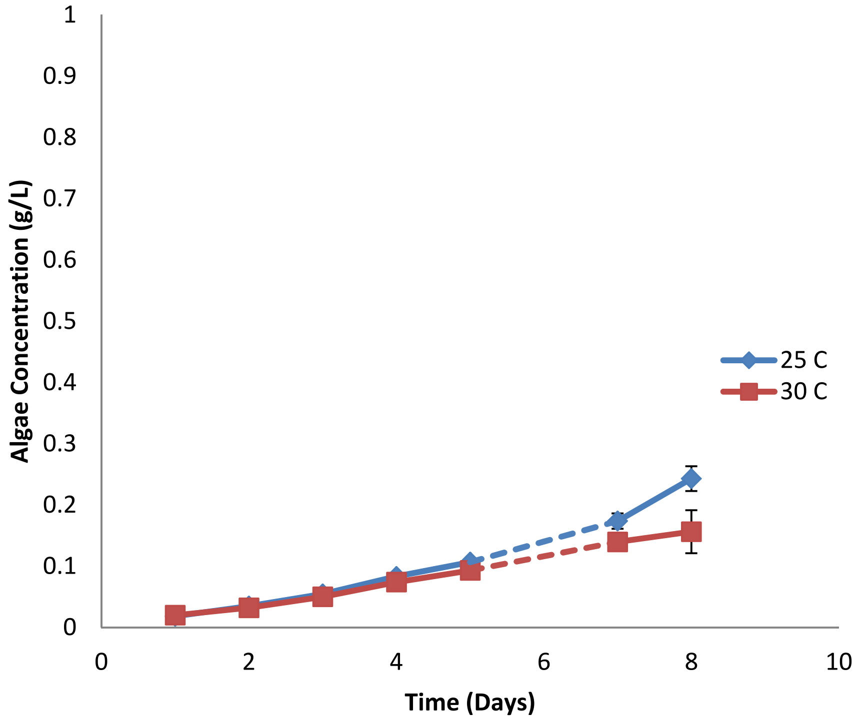

Figure 4: Growth curve of algae at 25 °C and 30 °C growth conditions (Preliminary Experiment 1)

Temp °C	Δ[m] g/L
25	0.0285 ± 0.009
30	0.0144 ± 0.005

Table 1: Average mass gained per day (Preliminary Experiment 1)

Temp °C	[m] (g/L)
25	0.2800 ± 0.070
30	0.1667 ± 0.040

Table 2: Average final biomass concentrations (Preliminary Experiment 1)

Preliminary Experiment 2 – 25 °C and 30 °C

Temp °C	Δ[m] g/L
25	0.0099 ± 0.003
30	0.0093 ± 0.017

Table 3: Average mass gained per day (Preliminary Experiment 2)

Temp °C	[m] (g/L)
25	0.4938 ± 0.066
30	0.4805 ± 0.037

Table 4: Average final biomass concentrations (Preliminary Experiment 2)

Temp °C	g/L*Day (lipids)
25	0.0106 ± 0.002
30	0.0106 ± 0.002

Table 5: Lipid yield (Preliminary Experiment 2)

Growth Experiment Overview: The growth experiment is identical to the preliminary experiments in objectives and design with the exception of temperature selections. Samples of algae were grown and monitored to observe the effects of 25 °C and 35 °C on growth rates and biomass. Afterward, the Folch lipid extraction method was applied to the biomass at 25 °C agitation temperature to measure the effects of the 25 °C and 35 °C growing temperature on lipid concentrations.

Growth Experiment – 25 °C and 35 °C

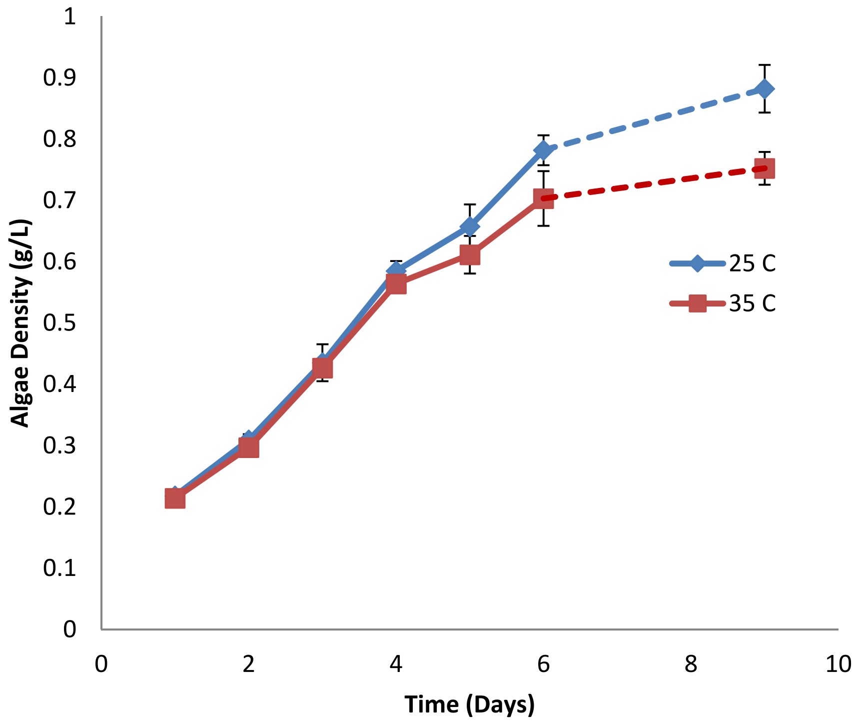

Figure 5: Growth curve of algae at 25 °C and 35 °C growth conditions (Growth Experiment)

Temp °C	Growth Rate (g/L*day)
25	0.1496 ± 0.030
35	0.1373 ± 0.012

Table 6: Maximum specific growth rate of algae (Growth Experiment)

Temp °C	Δ[m] g/L
25	0.0682 ± 0.002
35	0.0642 ± 0.004

Table 7: Average mass gained per day (Growth Experiment)

Temp °C	[m] (g/L)
25	0.7602 ± 0.019
35	0.7246 ± 0.040

Table 8: Average final biomass concentrations (Growth Experiment)

Temp °C	% Lipids/Mass
25	0.1545 ± 0.004
35	0.1773 ± 0.006

Table 9: Lipid concentrations per mass of total algae (Growth Experiment)

Temp °C	g/L*Day (lipids)
25	.0172 ± 0.001
35	.0197 ± 0.001

Table 10: Final average total lipid yield from algae (Growth Experiment)

Extraction Experiment Overview: The Extraction Experiment was identical to the Growth Experiment in that triplicate samples of algae were grown and monitored to observe the effects of 25 °C and 35 °C on growth rates and biomass. The difference in the lipid extraction portion of the experiment was that the Folch lipid extractions were performed at temperatures of 25 °C, 35 °C, and 50 °C. The opportunity was also taken during the Extraction Experiment to measure growth variables that had not yet been observed by the Growth Experiment, such as nutrient uptake and pigment analysis by fluorescence. These new measurements did not alter the procedure, but were simply valuable tests and observations included in the growth phase.

Extraction Experiment – 25 °C and 35 °C

Temp °C	% Lipids/Mass
25	0.1778 ± 0.013
35	0.2240 ± 0.026

Table 11: Lipid concentrations per mass of total algae (Growth Experiment)

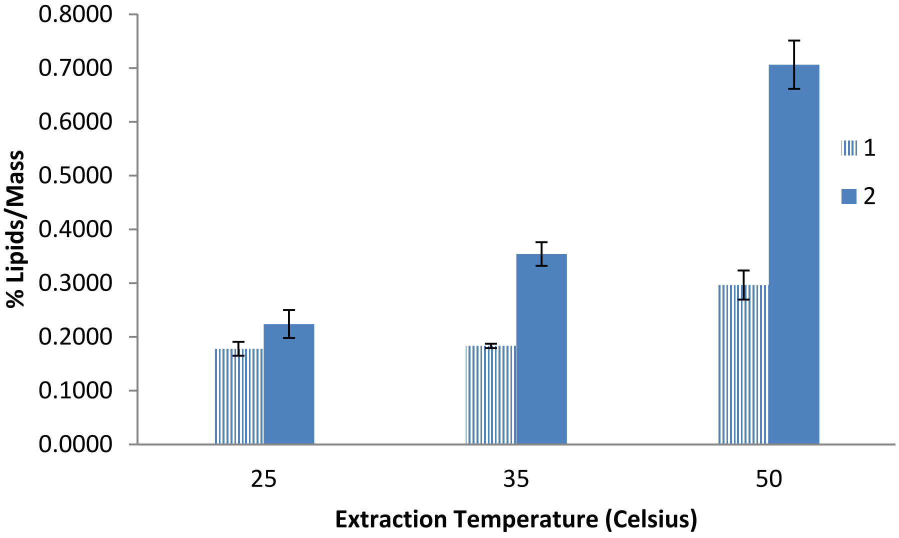

Figure 6: Lipid extraction efficiency of two samples at various extraction temperatures (Extraction Experiment)

Preliminary Experiments 1 and 2:

According to the growth chart of Preliminary Experiment 1 (Figure 1), the samples at 25 °C appeared to grow at faster rates when compared with the 30 °C samples. The 25 °C samples appear to still be in the exponential phase when the 30 °C samples reached stationary phase and the experiment ended. At this point, between Days 7-8, the 25 °C samples exhibited an apparent maximum rate of growth. However, this value can only be correlated to absorbance values since volume of the samples was not measured. As seen in Table 1, the 25 °C samples exhibited an apparent higher average mass gained per day. However, no significant differences were found between temperature and average mass gain. (p=0.099).

In Preliminary Experiment 2, the algae grew steadily past the usual one-week harvest time and exhibited stable growth for three weeks. Again, no significant differences were found relating temperatures and average mass gain (p=0.776), nor temperature and lipid yields (p=0.999).

Growth Experiment:

In the Growth Experiment, the growth curve (Figure 5) for the 25 °C samples diverged from the 35 °C samples around day 3 and exhibited higher algal densities for the remainder of the exponential growth phase until harvest. These differences appear minute, and statistical analysis shows no that there are no significant differences between growth and temperature (p=0.342). The fastest growth rate observed was 0.1496 ± 003 g/L*day. In this experiment, the average mass gained per day again appears slightly higher at 25 °C. However, the statistical analyses did not show any significant differences of average mass among the two temperatures 25 °C and 35 °C (p=.342). Though no difference in mass gain can be attributed to temperature, ANOVA supports temperature's role in lipid concentration differences (p=0.019). A subsequent t-test showed that 35 °C resulted in higher lipid yields than 25 °C (p=0.033).

Extraction Experiment:

During the Extraction Experiment, there was clearly some variable affecting the growth of the 35 °C samples, which exhibited little to no growth, as the average mass gain was an order of magnitude smaller than any other average mass gain values throughout all experiments. From the data in Table 11, it appears again that slightly higher lipid concentrations resulted from the growth at 35 °C. ANOVA supported temperature's role in lipid yield differences (p=0.007). A t-test confirmed that growth at 35 °C again yielded higher lipid concentrations than growth at 25 °C (p=0.007). Because of the failed growth of the 35 °C samples, the nutrient uptake data was omitted, as accurate comparison could not be made from this experiment.

Different extraction temperatures of 25 °C, 35 °C, and 50 °C were run for both samples grown at 25 °C and 35 °C. As seen graphically in Figure 6, the Group 1 samples represent cultures grown at 25 °C and the Group 2 samples represent cultures grown at 35 °C. At each extraction temperature, Group 2 extractions resulted in higher lipid yields than Group 1 yields. For Group 1, ANOVA supported extraction temperature's role in lipid recovery (p=0.0006). Subsequent t-tests confirmed that extraction at 50 °C yields higher recovery than extraction at 35 °C (p=.048), but the differences between extractions at 25 °C and 35 °C are not significant (p=0.278). For Group 2, ANOVA confirmed temperature's role in lipid recovery (p=.0000047). t-tests revealed that differences between

25 °C and 35 °C were significant (p=0.0014) and that differences between 35 °C and 50 °C were significant also (p=0.0007).

Discussion

Preliminary Experiments 1 and 2: Average mass gain data as well as one complete set of lipid yield data were successfully collected from the preliminary experiments, and statistical analyses found no significant differences between algal growth and lipid yield for samples grown at 25 °C and 30°C. In Preliminary Experiment 1, only initial volume was measured and volume was not compensated for evaporation. Without a final volume, lipid yield along with other density dependent values, could not be calculated. In Preliminary Experiment 2, though the initial and final volumes were measured, the volumes again could not be compensated for evaporation. Because of the inconsistencies of evaporation, it was decided that volume must be kept constant to keep all future experiments comparable. For example, in Preliminary Experiment 2, it took 22 days for the samples to reach steady state. Though the final volumes were measured and corrected, it is highly probable that steady state had been reached long before 22 days, but the constant evaporation artificially increased the optical density of the samples. Maintaining constant volume would prevent this in the future and would allow for accurate averages and standard deviations to be calculated for each growth data point.

Growth Experiment: The procedures established during preliminary experiment were successfully implemented for this experiment. Volumes were kept constant, all materials were kept sterile, and all corresponding growth parameters were accurately measured. During a span of eight days, this experiment resulted in rapid growth of both temperatures. Though slightly higher growth seemed to favor 25 °C, ANOVA found the differences to not be significant (p=0.342). Thus, it can be concluded from the data that for our native strain of algae, the temperature range of 25-35 °C has negligible effect on algae growth rates. Thus, it can be concluded that the optimal temperature range of our native strange has larger limits than studied here, and thus would not exhibit dramatic differences in biomass unless pushed to more extreme temperatures.

For this experiment, significant differences in lipid concentrations were found between samples grown at 35 °C and 25 °C. For a batch culture, this is normal, as limiting nutrients have been shown to increase lipid accumulation (Xin, 2010). To test the effect of temperature on the co-culture metabolism and discover if nutrients were in limiting quantities, initial and final nitrate readings were taken later during the Extraction Experiment.

Extraction Experiment: During the growth phase, the 25 °C group exhibited fair growth, but the 35 °C group exhibited very little growth. The cause for the lack of growth at 35 °C is unknown, as the final nitrate levels found at harvest time were not low enough to be considered limiting (N = 32.36 mg/L). The 25 °C group utilized more nitrate and phosphate per day and also did not deplete either nutrient by harvest time. Thus, it must be concluded that some mechanism other than nitrogen depravation was influencing both the lack of growth and lipid accumulation of the 35 °C samples. A possibility is that the lipids were already present in the sample, and values remained relatively stable, or even declined for the four days until harvest. An accurate way to objectively measure this in the future would be to perform a lipid analysis before and after growth to measure true lipid gain as well as biomass gain.

Though the growth of the 35 °C group in the Extraction Experiment was stunted, lipid concentrations were higher at 35 °C than 25 °C (p=0.009). It was surprising to discover that the chlorophyll a levels from both temperature ranges declined below initial levels. This may be due to chlorophyll degradation or experimental error.

Cost Analysis: A brief energy balance calculation below has been applied to the lipid extractions run at 35 °C and 50 °C to determine whether or not increased input energy for lipid extraction would result in profit for biodiesel production.

Samples 25 °C				
Extraction Temperature	Additional Energy Input (kJ)	m lipids (g)	Lipid energy (kJ)	Additional Gain in Energy (kJ)
25	0.0000	0.0081	0.2413	
35	0.9546	0.0084	0.2503	0.0089
50	2.3865	0.0139	0.4141	0.1639

Samples 35 °C				
Extraction Temperature	Additional Energy Input (kJ)	m lipids (g)	Lipid energy (kJ)	Additional Gain in Energy (kJ)
25	0.0000	0.0035	0.1043	
35	0.9546	0.0057	0.1698	0.0655
50	2.3865	0.0113	0.3367	0.1668

Tables 12 and 13: Charts display energy balance between additional input and returns from increased lipids

As seen from the tables above, though t-tests indicate greater lipid yields available for extraction at higher temperatures, a quick calculation proves that supplying excess energy to the lipid extraction process is cost-inefficient. At a glance, the argument can be made that if we were to scale up operations and deal with larger algal biomasses, the amount of lipids recovered would be greater, and thus maybe profitable. However, one must realize that in lipid extraction, solvents must be added in certain proportions to tissues. In this Folch method, the solvents added are 18X the tissue volume. Thus, the larger the algal biomass processed for lipid extraction, the larger the volume of solvent that we must heat up. There is no maximization point, as the curves will never intersect between additional energy input and additional gain in energy. A possibility, however, would be to try and use freely available energy that need not be purchased to optimize lipid extraction gains at higher temperatures. For example, hot waste water from a waste stream could provide the adequate heating in nighttime operations, or solar concentrators mounted around the agitation tanks could provide the necessary energy during the day. However, a new cost-benefit analysis must be performed to ensure that the extra revenue gained from additional lipids extracted would cover the initial capital requirement for infrastructure necessary to harness and direct the free/low cost energy to the extraction process and also the maintenance costs over the lifetime of the plant.

Conclusion: The data indicates that samples grown at temperatures of 35 °C consistently result in higher lipid concentrations when compared to 25 °C. Furthermore, the growth rates resulting from 35 °C are very similar to that of 25 °C. This is good news for outdoor ponds in Louisiana, where the average temperature of the growing season ranges between 22-32 °C, because the local algal ponds and raceways can count on steady growth and lipid yields within the seasonal growing period when utilizing this Louisiana co-culture. Louisiana's abundance of sunshine and large bodies of water (which have a high specific heat) should be explored to create methods of collecting heat during the daytime and transferring the heat to the lipid extraction process where higher temperatures were proven to substantially increase lipid yields.

Acknowledgements

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Cost Analysis

Appendix

Energy Content of Lipids	
29.794264	Kj/g
29794.264	joules/gr
7121.00	calories/g

1 calorie = 4.18400 joules

Folch Solvent Mixture

	density	**Vol**	**Cp (KJ/Kg*K)**	**Vol/vol**		
chloroform	1.4830	20.0000	1.0500	0.5263	0.5526	
methanol	0.7918	10.0000	2.5100	0.2632	0.6605	
water	1.0000	8.0000	4.1860	0.2105	0.8813	
		38.0000			2.0944	Cp*mix
	total weight	45.5780	grams			
		0.0456	kg			

Extraction Samples 25	m*Cp*(T-T)			
Extraction Temperature	Additional Energy Input (kJ)	m lipids recovered(g)	Total lipid energy (kJ)	Additional Gain in Energy from Lipids
25	0.0000	0.0081	0.2413	
35	0.9546	0.0084	0.2503	0.0089
50	2.3865	0.0139	0.4141	0.1639

Extraction Samples 35	m*Cp*(T-T)			
Extraction Temperature	Additional Energy Input (kJ)	m lipids (g)	Total lipid energy (kJ)	Additional Gain in Energy from Lipids
25	0.0000	0.0035	0.1043	
35	0.9546	0.0057	0.1698	0.0655
50	2.3865	0.0113	0.3367	0.1668

Maximum Specific Growth Rate (Extraction)	
Temp °C	Growth Rate (g/L*day)
25	0.0554 ± 0.009
35	0.0187 ± 0.004

Maximum Specific Growth Rate (Growth)	
Temp °C	Growth Rate (g/L*day)
25	0.1496 ± 0.030
35	0.1373 ± 0.012

Nutrient Uptake (Extraction)						
Temp °C	ΔN	ΔN/day	N Final	ΔP	ΔP/day	P Final
25 (4)	9.9867	1.9973	20.9867	9.2267	1.8453	8.4333
35 (4)	5.1200	1.2800	32.3600	5.9200	1.4800	10.9733

Mass Gain (I)	
Temp °C	Δ[m] g/L
25	0.2283 ± 0.070
30	0.1150 ± 0.040

Final [Biomass] (I)	
Temp °C	[m] (g/L)
25	0.2800 ± 0.070
30	0.1667 ± 0.040

Mass Gain (2)	
Temp °C	Δ[m] g/L
25	0.2172 ± 0.066
30	0.2038 ± 0.037

Final [Biomass] (2)	
Temp °C	[m] (g/L)
25	0.4938 ± 0.066
30	0.4805 ± 0.037

Mass Gain (3)	
Temp °C	Δ[m] g/L
25	0.6135 ± 0.019
35	0.5780 ± 0.040

Final [Biomass] (3)	
Temp °C	[m] (g/L)
25	0.7602 ± 0.019
35	0.7246 ± 0.040

Mass Gain (4)	
Temp °C	Δ[m] g/L
25	0.1933 ± 0.031
35	0.0167 ± 0.006

Final [Biomass] (4)	
Temp °C	[m] (g/L)
25	0.45666 ± 0.031
35	0.15666 ± 0.006