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EFFECTS OF ENVIRONMENTAL FACTORS ON THE GROWTH OF ALGAE FOR BIOFUEL PRODUCTION IN A HOUSEHOLD –BASED ALTERNATIVE ENERGY SYSTEM

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ABSTRACT

The efficiency and cost effectiveness of using algae to create biofuel has been much debated in recent years. A household-based biofuel system that uses sewage as a medium in which to grow mixed algae cultures is a possible solution to decreasing the production and transportation costs of biofuel. Aside from the manufacturing issues involved, it is important to determine what variables most affect the growth of algae and the production of biofuel. This study presents the results of an ANOVA-based set of experiments that evaluate the effect of several variables on algae growth in wastewater. While much work remains to be done, these results will help determine the optimal environmental conditions for a household-based biofuel system that utilizes mixed algae cultures.

INTRODUCTION

Current practices of producing oil from algae are costly and mostly ineffective [1-2]. Research in this area is focused mainly on the use of pure cultures to produce biofuel. Many of these cultures consist of Genetically Modified Organisms (GMOs). These GMOs that are utilized in current practices are not only costly, but also potentially hazardous to the environment when produced on a large scale [3-4].

The study of the effects of stress [5] and light intensity, as important factors [6], have been conducted. The latter related the light intensity to the growth rate by a logarithmic relationship derived by Sorokin and Krauss [7]. The effects of pH-CO_2-bicarbonate systems on the distribution of fresh water algae, experimentally explored by Moss, [8] revealed the growth of oligotrophic algae at pH levels of 8.6-8.9 while eutrophic ones grew at levels above 9. The results were interpreted in terms of the presence of free CO_2 and bicarbonates in the media [8]. The influence of phosphorous on the algae blooms and the use of iron to mitigate such influence are discussed by Lathrop et al. [9], Kara et al. [10] and Hoffman et al. [11]. The effects of Nitrogen levels [12] have also been studied by Horváth et al. on the quality of water.

A number of hypotheses have been developed over the last few decades concerning the growth of algae, in particular diatoms. These hypotheses state that the size of diatoms decreases and that their growth rate increases linearly as the temperature of their environment rises [13]. In addition to CO_2, algae consume P and N, the concentration of which may be critical in the species selection, growth rate and biofuel production rate. The growth of algae in waste water can produce clean water by lowering the levels of P and N [4,14,15]. This study investigates some of the effects described above along with the effects of stirring, shape of the containers, influence of light, hardness of water, and presence of silica on the growth of algae in waste water. The production of biofuel in closed systems that recycle water and nutrients alleviate several concerns raised in a recent National Research Council report by the Committee on the Sustainable Development of Algal Biofuels [3]. These concerns include the large amounts of water required (e.g. 3.5-3650 lit/liter of biofuel), large quantities of nitrogen consumed (1.5-4 g/lit of biofuel) and high levels of phosphorous (0.25-0.5g/lit of biofuel) needed to produce sufficiently large quantities of biofuel. To reach the targeted levels of biofuel production (39 billion liters annually), the US production of N should be increased by up to 107% and that of P should be increased by up to 51% [3].

The optimization of growth conditions for mixed microalgae cultures may help to develop an economic process for biofuel production on household rooftops, nearly 60 years after an earlier attempt to grow microalgae on the rooftops of MIT in the 1950s [15-19].

The study described in this paper focuses on a less common approach to algae biofuel production which involves the adoption of naturally-occurring mixed algae cultures that can be easily found in ponds and rivers. These cultures can grow in fresh water, waste water, and other media, adjusting to the environmental conditions by natural selection of the suitable algae species. Mixed cultures readily contain diatoms, known to produce large amounts of oil. The main challenge is to identify the critical factors that affect the biofuel production in desired environments.

The main objective of the study, more specifically, was to establish the effects of environmental factors such as light, nutrient level and temperature on algae growth in a mixed microalgae culture. The results will help optimize the growth condition for microalgae for biofuel production. The optimization of microalgae growth in waste water greatly accelerates the development of a household-based biofuel production system that is currently underway [25]. This system utilizes nutrient-rich waste water readily produced in the households and is capable of recycling water, P and N.

EXPERIMENTAL PROCEDURE

The DOE analysis that was conducted consisted of four parts: selection of variables and generation of an Analysis of Variance (ANOVA) table, preparation/growth of algae cultures, measurement of chlorophyll content, and analysis of results.

A. *Generation of ANOVA Table*

Two sets of experiments were conducted: one within deep test tubes and the other with shallow 10 ml tissue culture dishes needing no stirring. Six environmental factors, deemed to be effective in algae growth were selected and tested. These included light, temperature, silica (SiO_2), hardness ($CaCO_3$), stirring, and nutrients (Phosphorous and Nitrogen). In the second set of experiments with shallow dishes, stirring was omitted due to its impracticality. An L8 ANOVA table was constructed with 8 runs recording various effects as presented in Table 1.

Table 1 – ANOVA Table for Shallow-Dish Experiments

Run	Temp	Light	Silica	Hardness	P/N
1	-1	-1	-1	-1	-1
2	-1	-1	-1	1	1
3	-1	1	1	-1	-1
4	-1	1	1	1	1
5	1	-1	1	-1	1
6	1	-1	1	1	-1
7	1	1	-1	-1	1
8	1	1	-1	1	-1

The levels specified as 1 and -1 for temperature, light, silica, hardness and P/N refer to high and low levels for each of these factors. These levels are explained for each factor as follows: For temperature levels, 23 and 15 °C were used for levels 1 and -1 respectively. For light, two levels of high (290 lux) and low (180 lux) intensities were established where low intensity level was achieved by partially covering some of the test tubes. For silica and hardness factors, levels of 1 and -1 represented the presence or absence of silica (SiO_2) and calcium carbonate ($CaCO_3$). The high and low levels of nutrients were achieved by diluting the high-level nutrient samples with 2 mL of distilled water.

B. *Preparation and Growth of Algae*

The first set of experiments using test tubes, involved the filling of the tubes with 4 mL of sewer effluent, 4 mL of mixed algae culture, with or without SiO_2 and $CaCO_3$ according to the ANOVA table. Cultures requiring lower nutrition levels used 2 mL additional distilled water to dilute the P/N levels. Samples

of 1 mL were extracted from the tubes and tested for the chlorophyll contents at intervals of 2-day periods until the end of the sixth day.

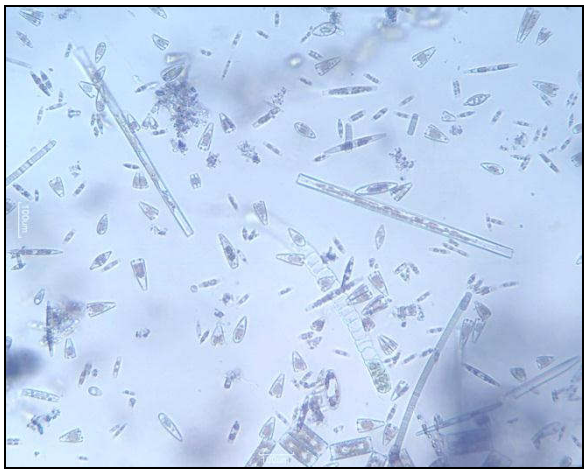

Figure 1 – Initial Mixed Algae Culture- Note all the algae in this picture are diatoms (mostly *Gomphonema and Synedra*) but the *cyanobacteria Oscillatoria* was also present in the original culture.

The second set of experiments using shallow dish cultures were conducted according to ANOVA table 1 (without stirring). A base of 4mL of mixed algae culture was used for each sample. The algae culture, which was collected from a local lake, contained various algae species including but not limited to: *Diatoma, Gomphonema, Nitzschia, Synedra, Navicula, Surirella,* and *blue green* algae. A picture of the initial algae culture is shown in Figure 1.

Four identical samples were prepared for each run in clear 10mL shallow containers. These samples were then placed in an environmental control chamber. The chamber had two compartments whose temperatures could be independently controlled. The compartments were set at 23°C and 15°C for the high and low temperature samples respectively. The light was restricted from 290 lux to 120 lux for the low light samples by covering them with a double layer of black window screen. High and low nutrient levels were controlled by the addition of sewage effluent. This wastewater, which was obtained from a local sewage treatment plant, was filtered to remove harmful organisms before addition to the algae samples. When silica and $CaCO_3$ were required they were added in a large enough quantity to ensure that they were not limiting factors. The experiments were conducted over a one week period. Two samples from each run were removed from the chamber on Day 4 for chlorophyll analysis. This helped track the growth of algae with time, making it possible to note any population changes half-way through the experiment. The remaining samples were left in the chamber till Day 7 when they were also removed for chlorophyll analysis.

C. *Chlorophyll Analysis*

For both set of experiments (deep tube and shallow dish cultures) samples were removed for analysis at various stages of experiments. After removal from the chamber each sample was filtered through 0.45 μm Millipore filter paper to separate the biomass from the liquid. The filter paper and biomass from each sample were then placed in a desiccator for a minimum of 24 hours. After this each sample was inserted into a 90% acetone solution to dissolve the filter paper and was then refrigerated for 24 hours. A spectrophotometer was used to measure the absorbance of each sample under wavelengths of 630, 647, and 664nm. These values were then used to calculate the total chlorophyll content per mL in each sample. This chlorophyll analysis provided a means of quantifying the growth of the algae in each sample over the one week period [20].

RESULTS

The results of the experiments conducted in this study show that main variables including light, temperature and nutrients have the greatest effect on the growth of algae. Sample data obtained from the experiments are presented in Table 2, as well as Figs. 2 and 3. The average chlorophyll content measured for each run on Day 7 is presented in Table 2 for each experimental run. Figure 2 is the probability plot for the residual effects illustrating the effects of various variables including the interaction of temperature and nutrients. The growth of algae within the first and second half time of growth is illustrated in Fig. 3. All these results correspond to shallow dish cultures with no stirring. The measured Chlorophyll values reported here are averages of two measurements on two identical samples for each run extracted at half-time and at the end of the experiments.

Table 2 – Average Chlorophyll Content on Day 7

Run #	Chlorophyll Content (μ grams/mL)
1	1.52
2	1.24
3	1.30
4	0.99
5	0.86
6	1.40
7	0.89
8	0.93

The results from Day 7 (Table 2) were then used to calculate the residual effects of each variable or combination of variables on algae growth (Table 3). A probability plot (Figure

2) was then created to determine which variables have the greatest effect on mixed algae growth over a one week period.

Table 3 – Residual Effects vs. Probability

Variables	Residuals	Probability (%)
Light/Temp	0.00	7.14
Temp/P/N	0.12	21.43
Hardness	2.12	35.71
SiO_2	2.24	50.00
Light	40.22	64.29
Temp	43.11	78.57
P/N	51.85	92.86

The growth pattern of the algae was plotted on a run chart (Figure 3) to observe the difference between the Initial, Day 4, and Day 7 average chlorophyll content per run.

The probability plot for the residual effects of each variable (Figure 2) indicates that the variables with the greatest impact on algae growth are light, temperature, and nutrients, since these points fall far from the trend line created by the other variables.

The run chart (Figure 3) indicates that three of the runs with the highest chlorophyll content at the end of the week initially decreased until Day 4 before they began to increase. This could have been due to the necessity of the algae to adapt to their new environment. Algae that could not do so died causing the initial decrease in chlorophyll content. All three of these runs had low light and two of them also had low temperature.

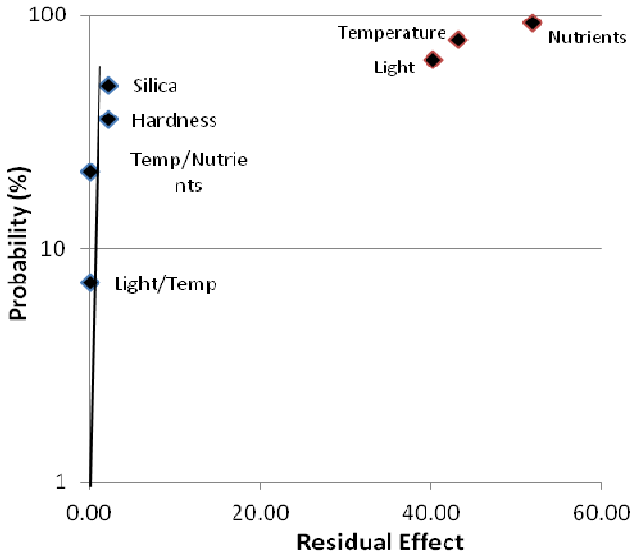

Figure 2 – Graph of Residual Effects vs. Probability

DISCUSSION

The results of this study, obtained for mixed algae, show greater effects for some of the main variables including light, nutrients and temperature. Deep-test tube experiments with stirring clearly show a greater dependency on light and light/temperature interaction. However, shallow dish cultures with no stirring indicate all three variables of light, temperature and nutrients are equally important. Results from these cultures do not point to any significance for hardness, silica or other interactions.

Nearly 90% of the deep test tube experiments exhibit a dual-mode behavior: A rapid increase in the microalgae population over a 2-day incubation period, which continues to further rise until Day4, followed by a noticeable decrease in the population by Day 6 of the runs. The exception is Run 6 where there is a high level of light but low levels of temperature and nutrients. In this particular situation, the growth rate is slow, and continues steadily upward until the end of the experiment.

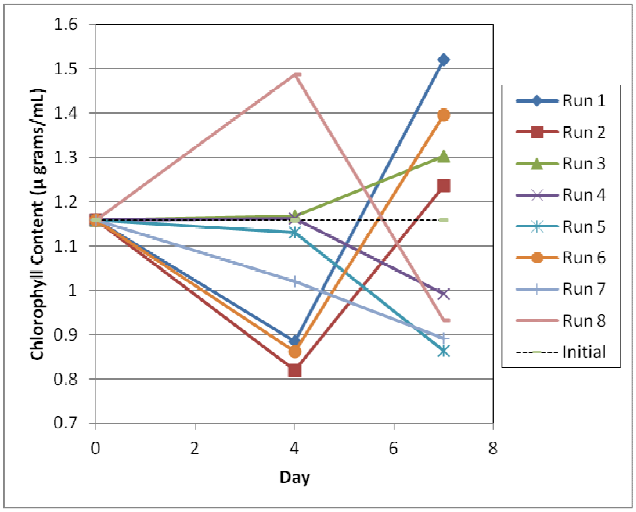

Figure 3 – 7 Day Change in Chlorophyll Content

Compared to the results of the test tube cultures, the shallow culture dish results indicate four runs with continued increase in the chlorophyll levels. Figure 3 indicates that three of the runs with the highest chlorophyll content at the end of the week initially decreased until Day 4 before they began to increase. This could have been due to the necessity of the algae to adapt to their new environment. All three of these runs had low light and two of them also had low temperature. It is therefore probable that the majority of the algae initially present in these cultures was used to higher levels of light and temperature. However, the algae best adaptable to the cool dark environments soon took over and then rapidly increased.

Of the four runs whose chlorophyll content remained constant or increased until Day 4, three of them had a dramatic decrease in chlorophyll by Day 7. This behavior indicates that the environment encountered by the algae in these samples may have required less adaptation. The algae would have been able to quickly use up all available nutrients; however, since more nutrients were not added the algae soon began to die off. Experiments using larger quantities of algae for longer periods of time should be conducted to better predict how the algae cultures will grow over time in a household wastewater based biofuel system. It is expected that the behavior seen in this experiment with chlorophyll levels increasing and decreasing over time as new species take over will continue. However, the rate at which this change happens (in this study approximately every four days) will vary due to the species and quantity of algae present.

CONCLUSIONS

The results of this study indicate that the variables with the greatest impact on the chlorophyll content of mixed algae cultures are light, temperature, and nutrients. While the chlorophyll analysis method that was used does not measure the algae lipid production, it does give an idea of how the growth of mixed algae cultures is affected by the presence or absence of light, temperature, silica, hardness, and nutrients.

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