

HARVESTING MICROALGAE (*CHLAMYDOMONAS REINHARDTII*) FOR
BIOFUEL PRODUCTION USING WASTEWATER TREATMENT
TECHNIQUES

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ABSTRACT

Microalgae are an important future source of biofuels because they produce high yields of oil and require far less acreage for cultivation than soybeans or corn. However, the current method of harvesting microalgae by centrifugation is inefficient and costly. My objective was to engineer an effective, efficient, and inexpensive way to harvest microalgae in preparation for biofuel production.

Chlamydomonas reinhardtii, the microalgae species chosen for this study, shows one of the greatest potentials as a source of biofuels because it yields approximately 50% oil by dry weight. Because microalgae are negatively charged microparticles that form into suspensions as a result of their negative surface charges, I used flocculation methods employed in wastewater treatments where high-cationicity chemicals are used to coagulate negatively charged microparticles.

A variety of high-cationicity compounds used in wastewaters treatment— $\text{Al}_2(\text{SO}_4)_3$, FeCl_3 , CaCO_3 , $(\text{NH}_4)_2\text{SO}_4$, and thirteen clarifloc polyacrylamides—were tested at a range of dosages and pH values to achieve 90% flocculation efficiency. The best flocculation efficiency of 99% was achieved by using Clarifloc polyacrylamide C-6288 at a dosage of 0.025 grams flocculant/grams dry microalgae and a pH of 3.00. This suggests that flocculation is a more effective and efficient method for harvesting *C. reinhardtii* microalgae than centrifugation.

INTRODUCTION

As the world's reserves of fossil fuels rapidly deplete, there is an increasing need for renewable energy sources—specifically biofuels. While potential sources of biofuels include corn and soybeans, research shows that algae have the most potential as a source of biofuels (1). My objective was to engineer an effective, efficient, and inexpensive way to harvest algae from suspensions in which the microalgae are grown in preparation for biofuel production.

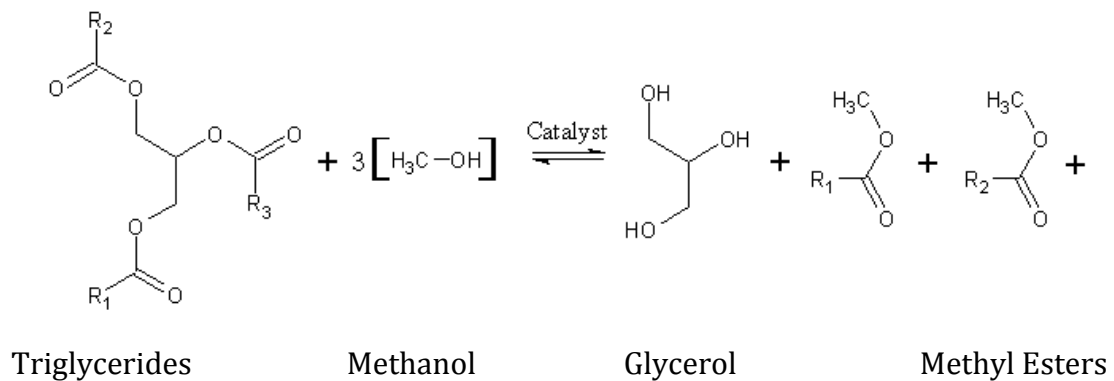
Algae show greater potential than corn or soybeans as a source of biofuel for several reasons. Some algae species yield up to 50% oil by dry mass compared to corn and soybeans that only yield on average 7% and 20% oil, respectively, allowing more biofuel to be produced per gram dry algae (2). In addition, algae require significantly less physical space for cultivation. Unlike corn and soybeans that require vast amounts of fertile farmland, algae can be grown in bioreactors in processing plants, using saltwater or wastewater (1). An algae processing plant yields 10,000 gallons of biofuel per acre of land per year, while corn and soybeans only yield 29 and 50 gallons, respectively (3). In order to produce enough corn biofuel to meet current fuel needs, over one million square miles of agricultural land must be cultivated—nearly half of the land in the entire USA—whereas, only fifteen thousand square miles of algae processing plants are required to produce the same volume of biofuel (4).

There are over 60,000 algae species; however, less than a thousand algae species yield high percentages of oil (5). Microalgae, known as diatoms, show the greatest potential as sources of biofuels because they yield up to 50% oil by dry mass (2). Microalgae are a class of algae that are extremely small in size, 5-30 μm .

Chlamydomonas reinhardtii is a species of microalgae that shows one of the greatest potentials as a source of biofuels because it yields approximately 50% oil by dry weight (1).

Currently, a multi-step process is used to produce biofuels from microalgae (Appendix A). First, microalgae must be cultivated in photobioreactors, which are growth systems that maximize photosynthetic potential (1). Then, microalgae are harvested from growth suspension using centrifugation, which yields concentrated microalgae paste. After the paste is dried and crushed into powder, oils (triglycerides) are extracted in methanol or hexanes using a soxhlet; these extracted triglycerides are then processed into biofuels, using esterification. Esterification involves the conversion of triglycerides into esters, which are biofuels (Figure 1).

Figure 1. Esterification reaction (http://en.wikipedia.org/wiki/Biodiesel_production)



Centrifugation uses centripetal force to agglomerate¹ suspended particles. Although centrifugation achieves a harvesting efficiency of 90%, centrifugation is expensive and inefficient (1). Centrifugation is expensive because microalgae are very small, so harvesting microalgae takes significant G-force. Centrifugation is inefficient because the size of the centrifuge limits the amount of microalgae than can be processed

¹ Agglomeration: To collect or form into a mass or group.

in one batch. Although effective, centrifugation is expensive, inefficient, and not feasible for large scale harvesting of microalgae; therefore, centrifugation is an unsuitable method to harvest microalgae in preparation for large-scale biofuel production (1). There are three alternative methods to centrifugation for harvesting microalgae:

- 1) Ultrasonic separation, in which standing sound waves create low pressure at nodes where microalgae concentrate (6);
- 2) Froth flotation, also termed foam fractionation, which uses high-pressure aeration in a microalgae suspension to create foam that captures microalgae particles and floats them to the surface (7); and
- 3) Flocculation, which uses chemical coagulants to cause microalgae to clump and settle (8, 9).

I chose to use flocculation because it can be used in a continuous system and requires no special equipment (1). I used information from *Water and Wastewater Technology* by Hammer and Hammer and ideas from a study by Lee et al. (2007) to engineer a method for harvesting microalgae. *Water and Wastewater Technology* explains that microparticles in wastewater form into suspensions as a result of their negative surface charges, and by adding high-cationicity² coagulants, negative surface charges are disrupted, causing microparticles in suspensions to flocculate and settle (8). The study by Lee showed that microalgae are negatively charged microparticles (9). Based on these two sources, I hypothesized that coagulants that show high-cationicity, which are used in wastewater treatment, could be used to flocculate microalgae. The

² The degree of positive charge on the coagulant

coagulants used in wastewater treatments that I used were $\text{Al}_2(\text{SO}_4)_3$, FeCl_3 , CaCO_3 , $(\text{NH}_4)_2\text{SO}_4$, and thirteen Clarifloc polyacrylamides (Appendix B).

I also used information from a study by Knuckey et al. (2006), which identified factors that affect flocculation of microalgae: dosage, pH, mixing rate, and mixing period (10). The Knuckey study showed that optimal flocculation factors vary depending on the microalgae species. However, the Knuckey study did not identify flocculation factors for *C. reinhardtii*. Based on the Knuckey study, I tested each coagulant at different dosages and at different pH levels to determine optimum flocculation factors for *C. reinhardtii*.

The main objective of my research was to engineer a more efficient and less expensive method of harvesting *C. reinhardtii* than centrifugation. My goals were to:

- 1) Determine if flocculation is an effective alternative to centrifugation for harvesting *C. reinhardtii* from suspension; and
- 2) Identify factors that optimize flocculation of *C. reinhardtii* in order to achieve harvesting efficiency that is similar to or better than centrifugation in preparation for biofuel production.

MATERIALS AND METHODS

Growing Algae: A 10-mL sample of *C. reinhardtii* was serially diluted to 10^{-8} M, smeared on Petri dishes containing growth media, and allowed to grow for two weeks. The microalgae were inoculated into 10 mL of autoclaved growth media and allowed to grow for three days. Then, the algae were transferred to 100 mL of autoclaved growth media. When the optical density of the algae reached 2.5 at 680 nm, the algae were transferred to 1 L of growth media. When the optical density of the micro algae reached

2.5 at 680 nm, the microalgae was transferred into 20-L coil-photobioreactors (Figure 2). Microalgae were allowed to grow to an optical density of 2.5 before harvesting.

Determining Efficacy of Harvesting by Flocculation: Four ionic compounds— $\text{Al}_2(\text{SO}_4)_3$, FeCl_3 , CaCO_3 , $(\text{NH}_4)_2\text{SO}_4$ —and thirteen polyacrylamides (Appendix B) were first tested at the dosage (1.0 gram/liter algae suspension) recommended in wastewater treatments (8). A range of other dosages were then tested to determine minimum dosage required for 90% flocculation efficiency. Flocculants were added to microalgae suspension at the point of the maximum mixing. The algae suspension was stirred at 50 hz for one minute and then at 10 hz for two minutes. After flocculated microalgae settled, optical density of the residual solution was measured at 680 nm (11).

Optimizing Flocculation Factors for C. reinhardtii: To adjust pH, 7.0 M HCl were added to lower pH to a range of 3-6, and 4.0 M NaOH were added to increase pH to a range of 8-11. To determine effects of mixing rates and periods, suspensions were mixed at rates between 20 and 60 hertz for 1-5 minutes, and 0-20 hertz for 5-10 minutes.

Data Analysis: Flocculation efficiency was determined using the following formula: $[(\text{OD}_o - \text{OD}_f) / \text{OD}_o] 100\% = \text{flocculation efficiency}$, where OD_o is the initial optical density at 680 nm, and OD_f is the optical density at 680 nm after flocculation. Flocculation efficiencies above 90% were considered effective (1).

RESULTS

The minimum dosages that achieved at least a 90% flocculation efficiency were 0.125 grams flocculant/grams dry microalgae for $\text{Al}_2(\text{SO}_4)_3$, 0.050 grams flocculant/grams dry microalgae for FeCl_3 , and 0.025 grams flocculant/grams dry

microalgae for Clarifloc polyacrylamides C-6244, C-6287, C-6288, and C-9545 (Table 1, Appendix C). At no dosage were CaCO_3 or $(\text{NH}_4)_2\text{SO}_4$ effective. A flocculation efficiency of 95% was achieved using FeCl_3 at a dosage of 0.050 grams flocculant/grams dry microalgae at a pH of 3.00 (Figure 3). (Figure 3-6 are seen in Appendix C.) A flocculation efficiency of 94% was achieved using $\text{Al}_2(\text{SO}_4)_3$ at a dosage of 0.13 grams flocculant/grams dry microalgae at a pH of 7.00 (Figure 3). A flocculation efficiency above 94% was achieved by using Clarifloc polyacrylamide C-6244, C-6288, and C-9545 at a dosage of 0.025 grams flocculant/gram dry microalgae at a pH 3.00 (Figure 4). None of the polyacrylamides were effective at pH 7.00 (Figure 5). However, when used in combination with $\text{Al}_2(\text{SO}_4)_3$ at pH 7.00, Clarifloc polyacrylamides C-6244, C-6287, and C-9495 achieved a flocculation efficiency above 90% (Figure 6). A flocculation efficiency of 99% was achieved by Clarifloc polyacrylamide C-6288 at a dosage of 0.025 grams flocculant/gram dry microalgae at a pH 3.00 (Figure 4). Optimum mixing characteristics were determined to be 50 hertz or more for one minute, and then 10 hertz for at least two minutes (Table 2, Appendix C).

DISCUSSION

Results supported my hypothesis that high-cationicity coagulants used in wastewater treatments effectively destabilized *C. reinhardtii* in suspension, causing the microalgae to flocculate. Chemicals that were most effective in flocculating *C. reinhardtii* were $\text{Al}_2(\text{SO}_4)_3$, FeCl_3 , and Clarifloc polyacrylamides C-6244, C-6287, C-6288, and C-9545 (Figure 5, 6). Among these, Clarifloc Polyacrylamide C-6288, which has a cationicity of 80%, showed 99% flocculation efficiency (Figure 3).

My goal to determine specific factors that optimized flocculation of *C. reinhardtii* was successful. The most effective factors for flocculating *C. reinhardtii* were 0.025 grams flocculant/gram dry microalgae, using Clarifloc Polyacrylamide C-6288 at pH 3.00.

CONCLUSION

The result of 99% flocculation efficiency for Clarifloc Polyacrylamide C-6288 at pH 3.00 suggests that flocculation is particularly effective for harvesting microalgae. Since there is no limitation on per batch volume when harvesting microalgae using flocculation, results suggest that harvesting by flocculation may be significantly more efficient than harvesting by centrifugation. Furthermore, use of polyacrylamides and disposal of polyacrylamides do not pose environmental risks since the polyacrylamides used in my study are the same as those used in wastewater treatments.

I tested flocculation using only one species of microalgae, *C. reinhardtii*. Since the Knuckey study showed that optimum flocculation factors vary depending on microalgae species (12), attempts to use the flocculation factors that I determined may not be as effective for other species as they were for *C. reinhardtii*. Work should be done to determine optimum flocculation factors for other high-oil yielding species of microalgae, since other species may also prove to be viable candidates for biofuel production.

My research provides new information that will increase the efficiency of large-scale harvesting of microalgae for biofuel production. Increasing harvesting efficiency will decrease costs, making microalgae an even more viable alternative to fossil fuels.

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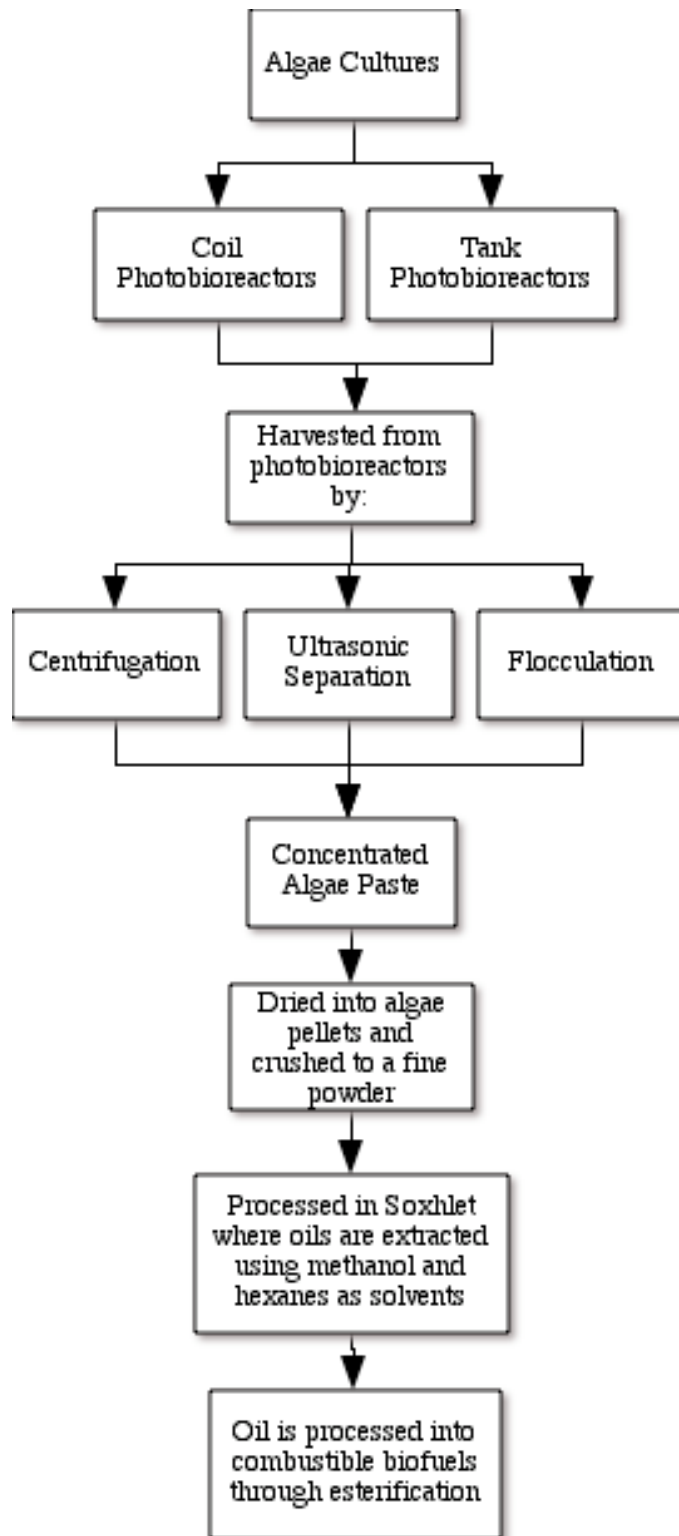
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APPENDIX A. ALGAE BIOFUEL PRODUCTION PROCESS



(Diagram by author)

APPENDIX B. POLYACRYLAMIDE INFORMATION

Polyacrylamide number used in paper	Polyacrylamide name	Cationicity (%)
1	Clarifloc Polyacrylamide C-6227	20 %
2	Clarifloc Polyacrylamide C-6228	20 %
3	Clarifloc Polyacrylamide C-6242	20 %
4	Clarifloc Polyacrylamide C-6244	40 %
5	Clarifloc Polyacrylamide C-6247	40 %
6	Clarifloc Polyacrylamide C-6262	40 %
7	Clarifloc Polyacrylamide C-6266	60 %
8	Clarifloc Polyacrylamide C-6267	60 %
9	Clarifloc Polyacrylamide C-6286	60 %
10	Clarifloc Polyacrylamide C-6287	80 %
11	Clarifloc Polyacrylamide C-6288	80 %
12	Clarifloc Polyacrylamide C-9530	80 %
13	Clarifloc Polyacrylamide C-9545	60 %

APPENDIX C. TABLE 1-2 AND FIGURES 3-6

Flocculant	Dosage (gram flocculant/gram dry microalgae)	
	Minimum	Maximum
$\text{Al}_2(\text{SO}_4)_3$	0.125	0.50
FeCl_3	0.050	0.50
CaCO_3	0.125	1.00
$(\text{NH}_4)_2\text{SO}_4$	0.125	1.00
All thirteen polyacrylamides	0.025	0.25

Figure 3. Flocculation efficiency for four ionic compounds at pH 7.00 and pH 3.00

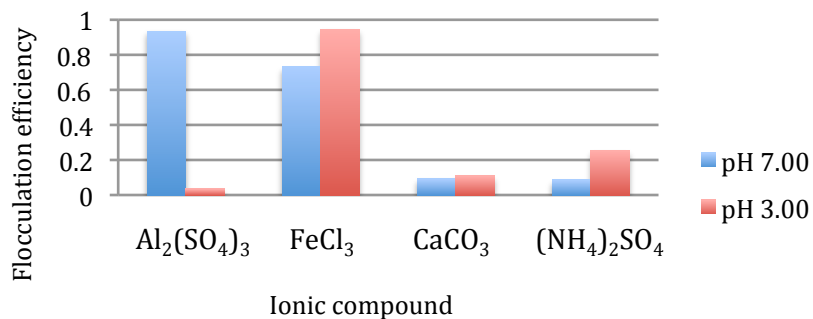


Figure 4. Flocculation efficiency for polyacrylamides at pH 3.00

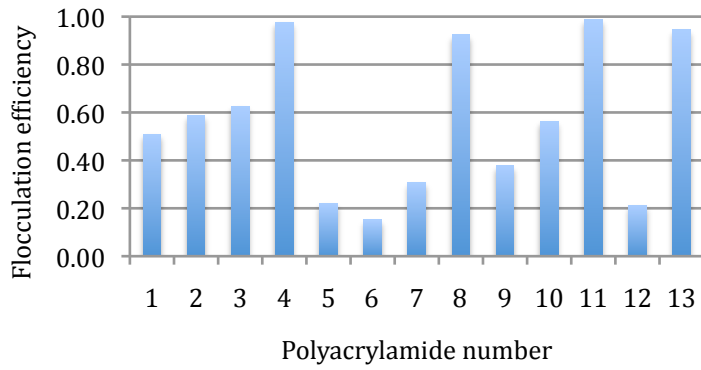


Figure 5. Flocculation efficiency for polyacrylamides at pH 7.00

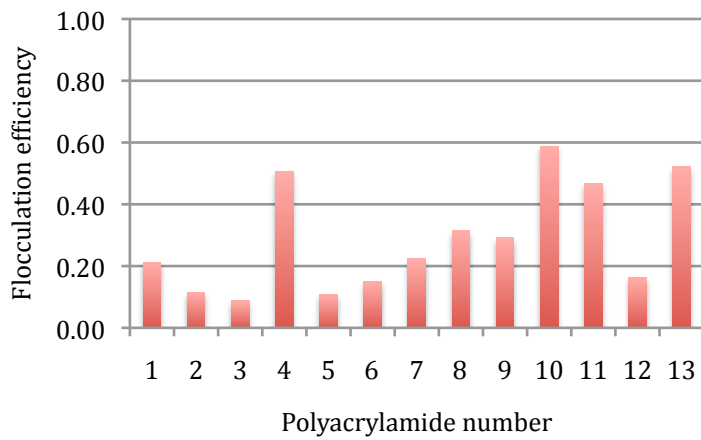


Figure 6. Flocculation efficiency for polyacrylamides in combination with $Al_2(SO_4)_3$ at pH 7.00

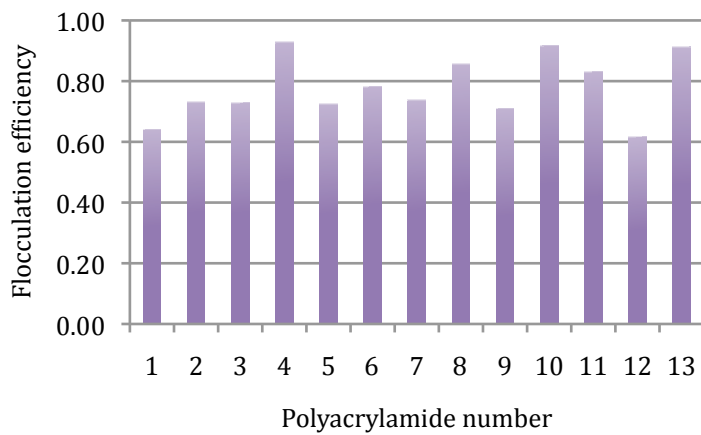


Table 2. Mixing rates

Mixing part one		Mixing part two		Rank order of mixing effectiveness
Rate (Hz)	Period (s)	Rate (Hz)	Period (s)	
10	60	10	360	6
20	60	10	300	5
30	60	10	240	4
40	60	10	180	3
50	60	10	120	1
60	60	10	60	2