The Effect of Glycerol Addition on Growth of *Scenedesmus* in Landfill Leachate

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Abstract

Microalgae have a rapid growth rate and possess the ability to accumulate intracellular lipids. Algae also have significant potential for wastewater bioremediation. Algae can be grown in three modes: autotrophic using light energy, heterotrophic using organic carbon sources, or mixotrophic using a mixture of both. Currently, heterotrophic and mixotrophic growth of algae is limited primarily due to the cost of procuring an organic carbon source. Glycerol is a waste by-product of biodiesel production. This resource could potentially be utilized as a carbon source to grow algae. The objective of this research was to investigate the effect of crude glycerol addition on biomass growth and lipid content of algae grown in landfill leachate. *Scenedesmus cf. rubescens*, a locally isolated strain with demonstrated ability to produce lipids, was selected as the test organism. *Scenedesmus cf. rubescens* was grown in Bold's Basal Medium. Algal growth experiments were conducted in 125 ml Erlenmeyer flasks (100 ml active volume). Subcultures were inoculated with exponentially growing mother cultures at a volumetric ratio of 20% (v/v). Cultures were illuminated at 300 µmol photons/m^2/s on a 12:12 photoperiod. All experimental trials were conducted in triplicate. Algal growth was monitored by spectrophotometry using absorbance at 680nm.

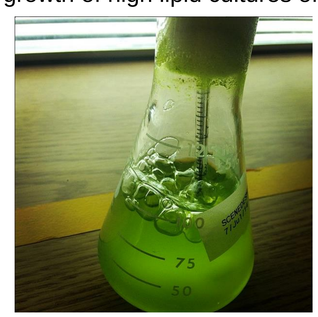
Introduction

The global agricultural resources available are not sufficient to satisfy the production of both biofuel and food on a level that would meet global demand. Green microalgae have been considered as biofuel feedstock that does not compete with the food supply because of their rapid growth rate and ability to accumulate intracellular lipids that can be converted to biodiesel [1]. Because large scale cultivation of algae would still require a large amount of resources, the has been research into whether wastewaters can be used as growing media for algal production. If done successfully, such algal cultivation would serve the dual purpose of wastewater treatment and biomass production in a single coupled system.

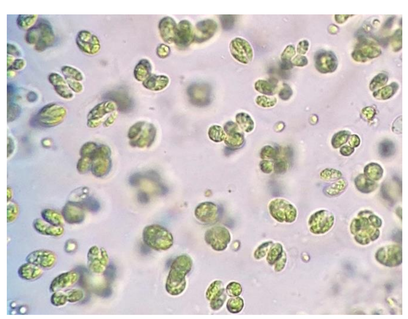
Scenedesmus cf. rubescens, has also been shown to be able to remove nitrate, phosphorus, and ammonia from wastewater [5] and have also been shown to grow on neutralized raw landfill leachate [3]. When grown under mixotrophic conditions, meaning they use both CO2 and glucose as a carbon source, Scenedesmus achieved high biomass production and total lipid content [4]. Additionally, when grown in media containing glycerol at a concentration of 10 grams per liter, they produced 10.8 times more lipids than Scenedesmus grown under purely autotrophic conditions [6].

Objectives

The objective of my experiment is to investigate weather the combination of landfill leachate and waste glycerol will create a good medium for the growth of high lipid cultures of Scenedesmus cf. rubescens.



Scenedesmus in 125ml Erlenmeyer Flask



Scenedesmus cf. rubescens viewed through a light microscope

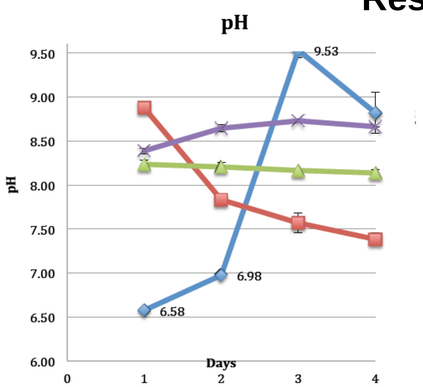
Methods

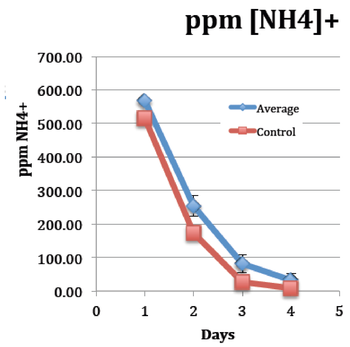
Four experimental groups were grown in triplicate:

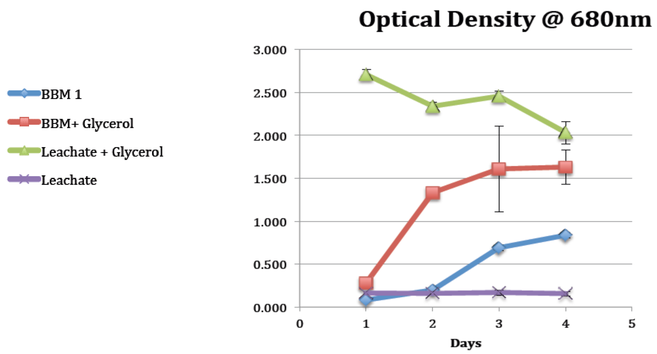
- Control: Scenedesmus cf. rubescens in Bold's basal medium
- Experimental group 1: Scenedesmusc cf. rubescens in Bold's basal medium supplemented with 10g/l waste glycerol.
- Experimental group 2: Scenedesmus cf. rubescens in landfill leachate neutralized with HCl.
- Experimental group 3: Scenedesmus cf. rubescens in landfill leachate neutralized with HCL and supplemented with 10g/l waste glycerol.
- One sterile flask of each leachate, leachate + glycerol, and Bold's basal medium + glycerol, was also kept as an abiotic control for each experimental group.

Solutions were prepared in 500 ml flasks, poured into their respective 125 ml flasks, and inoculated with a 20% solution of Scenedesmus from a mother culture prepared in Bold's basal medium. Cultures were setup with aeration through filtered pipet tubes and plugged with foam plugs to ensure sterility. Cultures were set at a lighting bay of an average photon flux of 300 µmol m-2 s-1on a 12:12 hour time cycle. The pH and optical density @680nm of all groups were measured daily for three days. Ammonium ion concentration (as a measure of algae growth and leachate remediation) was also measured daily on experimental group 2.

Results







Results (continued)

No significant algae growth was observed in any of the experimental groups. The pH and optical densities of all groups are shown the graphs below. Although the values in both graphs do fluctuate, these fluctuations align with the abiotic controls and therefore are indicative of physical rather than biological processes.

The amount of ammonium in the leachate groups decreased significantly; however, this was due to the physical sparging of ammonia molecules as the ppm of the abiotic control also decreased. A plot of the average of the experimental groups and the abiotic control can be seen in the graph entitled "ppm [NH4]+."

Failure of algae to grow was most likely due to an unfavorable pH, and the opaque character of the experimental solutions.

Conclusions & Further Research

The results of this experiment highlight the difficulties of working with waste products and wild strains of algae. Two barriers growing algae in this manner are the variability of the waste products, and fact that the behavior of the organism can not always be predicted.

More research into the characterization of the specific waste products to be used and the behavior of the chosen microalgae species is needed if bioremediation and biomass growth are to be achieved on landfill leechate and waste glycerol.

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