Executive Summary

From Microwave-Pretreated Sludge to Biodiesel Feedstock

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Summary

The results of our experiment suggested that microwave did not enhance sludge digestibility for bacterial growth. All of the TLC plates returned very minimal, suggesting little TAG accumulation after 4 days of cultivation. These results were consistent with the low bacterial growth, as indicated by the low DNA copy numbers as measured by real-time PCR.

Introduction

The handling of treated sludge at municipal wastewater facilities is a growing and complex problem. Currently, the sludge is disposed of in one of three ways; it is dewatered and buried in a landfill, incinerated, or used for land filling. This sludge also has the potential to be transformed into bioenergy because it consists largely of organic compounds.

In an effort to capture the most of the available bioenergy as possible, we have treated the sludge with different alkalis and exposed it to microwave irradiation. Presumably, these conditions will breakdown the present cells so that once added, the lipid accumulating bacteria, will consume that biomass.

After treatment we extracted the lipids and performed thin layer chromatography; then we extracted the DNA and performed a real-time qPCR. The results of which are discussed below.

PMA Dye

PMA dye is used to bind DNA of dead cells so that it can be used to differentiate from live cells. After we collected our 5mL samples each day, the sample was centrifuged for 10 minutes at 5000g. We then removed 4mL of supernatant from each sample and re-suspended the remaining 1mL. The remaining sample was transferred to a smaller vial, 2.5µL of PMA dye was added, and the vial was covered in aluminum foil to prevent light exposure. Next, the samples were placed on a rocker for 5 minutes before placing them on a shaker for 15 minutes. Finally, the samples were placed in the freezer until the DNA extraction was performed.

Lipid Extraction

Lipid extraction was performed on the remaining 80mL of sample on the final day of treatment. After the remaining solids were separated through centrifuging, 5mL of 1% NaCl solution was added to each vial, the sample was re-suspended, and then transferred to a glass vial. Next, a solvent was added to break down the cell walls and the samples were placed on a shaker for 18 hours. We then centrifuged the samples and removed the layer of water on top and retrieved 5mL of lipid solution on bottom. The lipid solution was then evaporated, leaving only the dried lipid. Finally, 100µL of a second solvent was added to complete the process.

Thin Layer Chromatography (TLC)

TLC was used in this experiment to visually show the accumulation of lipids (TAGs) in our samples. This was done by placing a TLC
plate in a hexane after 2µg of each sample, along with the standards, were placed along the bottom. The plate was removed from the hexane, dried, treated with 1M NaCl solution and then rinsed with amino black solution for 30 minutes. Our final result showed that lipid production was minimal only meeting the 1µ standard.

DNA Extraction
The purpose of DNA Extraction is to obtain DNA in a relatively purified form which can be used for further investigation, such as PCR. Most DNA extraction protocols consist of two parts. 1) A technique to lyse the cells gently and solubilize the DNA. 2) Enzymatic or chemical methods to remove contaminating proteins, RNA, or macromolecules. FastDNA™ SPIN Kit for Soil was used to isolate bacterial genomic DNA from soil samples in our experiment.

Real-Time PCR
The real-time PCR (Polymerase Chain Reaction) is a laboratory method used to amplify and quantify targeted DNA. It works the same way as normal PCR, except that real-time PCR uses primers to bind to genes of interest, which allows the polymerase to bind and begin copying the genes of interest. It quantifies by measuring the accumulation of amplified product after each cycle. Detection of DNA occurs through fluorescence. Hence, as the number of gene copies increases during a reaction, so will the fluorescence. Real-time PCR gives a look into each separate reaction and with the results given you will be able to know what reactions had the most DNA, compared to those that had the least.

DNA template is denatured at high temperatures and primers are added. After denaturing, temperatures are cooled and the primers bind to the desired target ends of the DNA sequence. Polymerase then commences to synthesize complementary strands of DNA until it ends a cycle. At the end of each cycle, double the amount of starting DNA is produced.

Based on the Log SQ and the number of cycles that took place, it seems as though NaOH-Autoclave accumulated the most DNA. This occurred because the NaOH Autoclave sample had the most copy numbers in the least amount of cycles. The second pretreatment that seemed to have the most accumulated bacteria is the KOH-2 microwave sample and the least favorable was the NAOH microwave sample.

Cost Analysis
Currently, there are no microwaves available to do our pretreatment on a large scale. With the little production of DNA produced by our selected method of pretreatment, it would not be feasible to use this method over incineration at $20.31 per wet ton, landfilling at $55 per wet ton or land application disposal methods at $77 per wet ton.

Conclusion
Microwave treatment with or without alkali were not effective to increase the digestibility of sludge. We hypothesized that the NaOH and microwave pretreatment would produce the most biomass with lipid. Minimal TAG accumulation was observed in all cases. To our surprise, the NaOH and autoclaved pretreatment, as the controls, produced the most biomass.