Problem Statement
The current disposal methods of sludge are not only costly, but they are increasing our carbon footprint. Our objective is to research beneficial ways to use this sludge. We aim to examine the feasibility of culturing TAG-accumulating bacterium as a biodiesel feedstock from various pretreated sludge.

Hypothesis
We hypothesize that NaOH + microwave pretreatment will greatly enhance the digestibility of sludge by the TAG-accumulating bacterium.

PMA Dye
Purpose: To use PMA dye to bind DNA of dead cells. The PMA modified DNA cannot be PCR amplified. This is used as a means to differentiate dead from live cells.
How it is Done: The collected sample was first centrifuged. After removing the supernatant, PMA dye was added to the remaining sample. The sample mixture was then incubated on a rocker, and then placed on a shaker.

Lipid Extraction
Purpose: To extract total lipids from the sample
How is it Done: On the final day, the remaining solution is centrifuged, combined with NaCl solution, re-suspended, and combined with a solvent that would break down the cell walls. From there, the sample was centrifuged again and the lipid was removed from the bottom layer. The lipid was evaporated until only the dry lipid remained.

DNA Extraction
Purpose: To obtain DNA for real-time PCR analysis.
How is it Done: First, the cells are gently lysed and the DNA is solubilized. Next the contaminating proteins, RNA or macromolecules are removed by an enzymatic or chemical process. For our experiment a FastDNA™ SPIN Kit for Soil was chosen to isolate bacterial genomic DNA from our samples.

Real-Time PCR
Purpose: To quantify DNA from live cells
How it is Done: Primers are used to bind genes of interest, this allows polymerase to bind and begin copying the genes. This data is quantified by measuring the accumulation of the amplified product after each cycle allowing for a comparison of DNA accumulated for each sample.

Conclusion
- NaOH + autoclave pretreatment proved to generate the most biomass of strain DP630, based results obtained from PMA-treated real-time PCR.
- NaOH + microwave pretreatment generated the least biomass of strain DP630.
- KOH + microwave pretreatment generated the second-most biomass of strain DP630.
- No significant TAG accumulation was detected in all cases.

AggiE-Challenge
Developing Next-Generation Wastewater Treatment Technologies
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