Human activities related to nitrogen have caused an overarching problem - an imbalance in the global nitrogen cycle. Through conventional wastewater treatment processes, mainly a combination of aerobic nitrification and anoxic denitrification processes, ammonia and other forms of nitrogen can be converted into nitrogen gas and return back to the nitrogen cycle. However, this approach not only requires significant input of energy and oxygen but also produces greenhouse gas like nitrous oxides during denitrification. A novel way to reduce energy and oxygen inputs while minimizing greenhouse production for nitrogen removal is necessary.

ANaerobic AMMonium-OXidizing bacteria (anammox) can convert nitrite and ammonium directly into nitrogen gas. Incorporating anammox into an engineered treatment system can be a promising strategy to remove nitrogen effectively and sustainably. The challenge of such an approach is that these bacteria grow extremely slow and require a much longer residence time than a traditional treatment process permits. The overarching research objective is to immobilize anammox and ammonia-oxidizing bacteria (AOB) in polyethylene glycol (PEG) gel beads in order to lengthen retention time and remove nitrogen with high efficiency.

To demonstrate the suitability of encapsulation in a wastewater setting, AOB, *Nitrosonoma Europea* pure strand, and nitrite-oxidizing bacteria (NOB), pure strand of *Nitrobacter Winogradskyi* (Fig. 1), were successfully entrained in PEG gel beads (Fig. 2). This phase of the project has been challenging, yet the preliminary results are promising. Removal of ammonium, creation/depletion of nitrite, and creation of nitrate achieved by the encapsulated bacteria was compared to that of suspended growth.

Analyzing with an ammonium probe, the encapsulated growth experiments decreased ammonium concentration by half in fewer than 10 days. The suspended growth experiment showed similar results in 5 days. Nitrite depletion was visualized using colorimetric test reagents. Based on color intensity after 10 days, the nitrite level decreased. Nitrate was measured using a Hach Kit and a spectrophotometer, proving that nitrate was produced. On the 20th of April Team Nitrogen presented the work completed this semester at the Engineering Project Showcase. It was a great pleasure to share the project with graduate students, professionals, and even high school students. Everyone that stopped by the booth showed interest and provided encouraging comments.

Goals for the next research phase include freezing AOB and NOB samples to preserve them for future use and quantifying the amount of cells alive in each reactor using DNA extraction methods and polymerase chain reaction (PCR). Determination of cell quantity will allow for a better comparison between the encapsulation method and suspended growth method tested.
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Team 1: Nitrogen removal

during this project. Additionally, continued research on anammox bacteria enrichment methods is necessary. Once anammox is successfully grown, it will be double encapsulated with AOB and incorporated into a reactor for the efficient removal of nitrogen from synthetic wastewater. The anticipated benefits of this approach are lower energy input, less oxygen demand, reduced creation of sludge, reduced production of greenhouse gas, and high removal rate of nitrogen.

Fig. 1.

Enriching AOB and NOB  Nitrite Depletion

Fig. 2.

Our Solution: Improve the Efficiency of Wastewater Treatment Processes with Encapsulated Bacteria

What do we mean by Encapsulation?
The bacteria are entrained in cross-linked polymer gel beads. The current method first encapsulated NOB into PEG squares. The NOB “squares” were then mixed with suspended AOB. Using a larger “bullet’ structure, the mixture was re-encapsulated in the PEG gel.

Possible benefits of using encapsulated bacteria?
Larger Retention Time
Production of Microbial Lipids from Wastewater

Faculty Advisor: Dr. Kung-Hui Chu

Introduction:

Biodiesel, a form of bioenergy, is a clean and renewable liquid fuel that can be made from materials that contain triacylglycerol (TAG), a major component in fats, oils and lipids. Activated sludge from wastewater treatment is a nutrient rich organic material (Fig. 1) and TAG accumulating bacteria are heterotrophic. Therefore, we hypothesize that it is possible to use sludge to grow TAG-accumulating bacteria, which can be used to produce biodiesel. The objective of this study is to investigate how the growth of Rhodococcus opacus strain PD630 is affected by applying different pretreatment methods-autoclaving and peracetic acid (PAA)-and altering the C/N ratio of the sludge.

Approach:

In this study, experiments are conducted in batch reactors and results are quantified using DNA extraction, PCR amplification, redox staining for live/dead cells, and visualization of TAG. Multiple trials have been conducted to verify result validity.

Results:

Visual results and all analytical analysis methods converged on the same conclusions:

- Peracetic acid is superior to autoclaving at treating sludge for cell growth (Fig. 2).
- Peracetic acid breaks down the cell walls of the sludge, making it easier for PD630 to absorb nutrients and produce lipids (Fig. 2).
- Cheese Whey aids in the accumulation of lipids (Fig. 2).

Further Analysis:

The results from this semester have paved the way for further analysis next semester, including determining why PAA is the most effective method of pretreatment for lipid accumulation, performing a life-cycle analysis, using batch-fed sequencing, and calculating the observed yield. These forward looking analysis methods focus on applying our results to the field by analyzing the effectiveness of the pretreatment methods in terms of money.