Effect of Nitrosomonas europaea Growth Rate on rRNA Degradation and Phenol Inhibition

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Introduction

Ammonia oxidizing bacterium (AOB) are a recent development for the removal of nitrogen in waste water treatment plants (WWTPs). However, these bacteria are very sensitive to being inhibited when they encounter other compounds commonly found in WWTPs such as aromatic hydrocarbons.

Nitrosomonas europaea, the model AOB, oxidizes ammonia as follows:

$$NH_3 + O_2 \rightarrow NO_2^-$$

***N. europaea*'s growth rate may be controlled through different retention times of a chemostat.** Therefore, a chemostat with a higher flow rate will produce faster growing bacteria, whereas a slower flow rate will produce slower growing bacteria.

Biofilms and suspended cells of *N.europaea* are suspected to be slow and fast growers, respectively. They demonstrate the following characteristics:

Biofilms
- More rRNA degradation due to slower growth rate
- Less inhibition by aromatic hydrocarbons

Suspended Cells
- Less rRNA degradation due to faster growth rate
- Greater inhibition by aromatic hydrocarbons

The goal of this study is to understand how the growth rate of N. europaea impacts the degradation of rRNA and the inhibition by phenol.

Methods

Twelve semibatch flasks are used to simulate a chemostat.

Cells were grown until they were past exponential growth phase. After this, in sets of triplicates, cells were removed and replaced with fresh media each day. Different volumes were replaced for each of the different sets; 2mL, 4mL, 10mL and 40mL. These correspond to different retention times as follows:

Transfer Quantity	Retention Time
2mL	100 Days
4mL	50 Days
10mL	20 Days
40mL	5 Days

In addition to transfers, cell density and NO_2^- production, via colorimetric assay, were measured daily. For the following 24 hours, the cells were placed in a shaker.

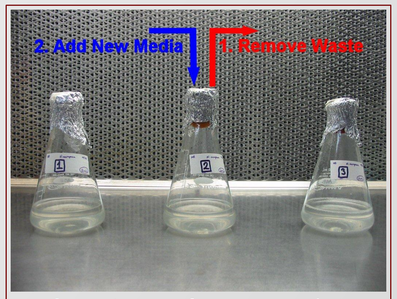


Figure 1: Transfers were made to individual flasks in a sterile hood.

Figure 2: Flasks were shaken overnight to provide oxygen.

Results

Development of Semibatch Chemostats

Transfers were made on the semibatch chemostats for a total of 44 days. Figures 3 and 4 depict the development of the cells in their NO_2^- production and the change in the concentration of cells for each group. **The large dip in both of the graphs is due to a lack of O_2 provided to the fastest growing cells.** Shaker speed was increased in order to correct this, which also led to the subsequent increase in protein concentration.

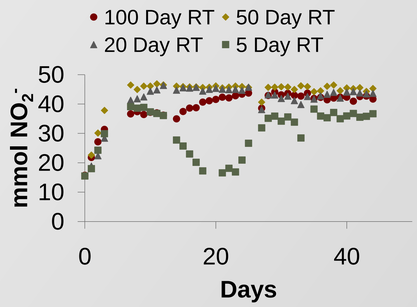
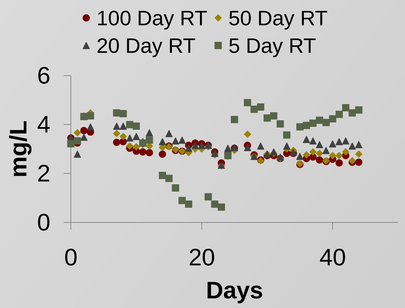


Figure 3. Average NO_2^- production for each group over time.

NO_2^- concentration increases during exponential growth phase, then levels out upon reaching steady state.

Figure 4. Average protein concentration for each group over time.

Protein concentration also increases during exponential growth phase, but decreases due to transfers and oxygen limitations before reaching steady state.

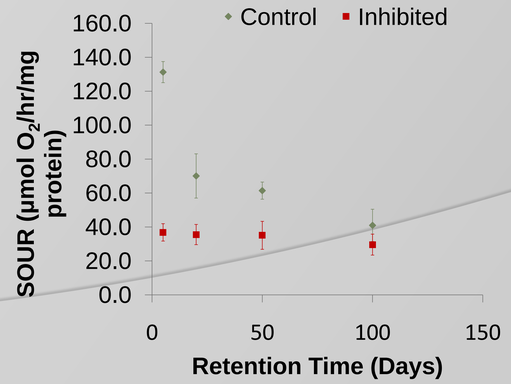


Phenol Inhibition Results

Once the cells reached steady state, phenol inhibition was tested by shaking the cells with and without 20μM phenol for 1h. Directly after shaking, activity was measured through the specific oxygen uptake rate (SOUR) method.

Figure 5. Oxygen uptake of cells with different retention times.

Cells at a faster growth rate, or lower retention time, are significantly more inhibited by phenol compared to the controls. Additionally, the activity of the inhibited cells does not change largely between cells of different growth rates.



RNA Results

RNA was extracted from the steady state semibatch chemostats. The quality was then measured through the use of an Agilent Bioanalyzer 2100.

Retention Time	Average RIN
100 Days	7.9 ± 1.6
50 Days	7.8 ± 0.88
20 Days	9.4 ± 0.17
5 Days	9.7 ± 0.23

Figure 6. Average RNA integrity numbers (RINs) for different retention times.

RINs quantify the quality of DNA. A lower RIN means RNA has become more degraded.

More rRNA degradation is apparent in samples of slower growth rate (higher retention time). As shown in Figure 7, there are larger areas under peaks and choppiness of rRNA before the 23s and 16s peaks that correspond to the rRNA quality.

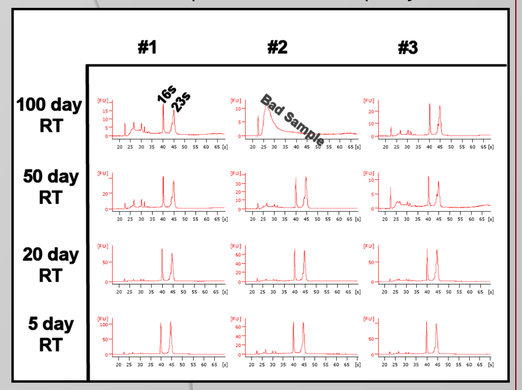


Figure 7. Electropherograms in terms of fluorescence units [FU] and time [s].

Conclusions

- **Simulating a chemostat of *N. europaea* culture through the use of a semibatch reactor is feasible and reaches a steady state (Figures 3 and 4).**
- ***N. europaea*'s inhibition by 20 μM phenol, compared to the controls, significantly increases as growth rate increases (Figure 5).**
- **Activity of *N. europaea* when inhibited by 20μM phenol does not vary largely between growth rates (Figure 5).**
- **Lower growth rates of *N. europaea* demonstrates more degradation of rRNA than that of higher growth rates (Figures 6 and 7).**

Acknowledgements

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