Application of Strain Tracking Techniques to Understand Wastewater Treatment Capabilities through Bioaugmentation

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ABSTRACT

Bioaugmentation is a promising technology for optimizing the microbial community responsible for wastewater treatment. Establishing the link between the performance improvements of a treatment plant and bioaugmentation holds the key for establishing the credibility of the bioaugmentation approach (D'Imperio et al., 2013). The study presented herein used a commercially available bioaugmentation product that did not have any nitrifying ability to bioaugment a battery of lab-scale, flow-through activated sludge reactors. The concentration of the bioaugmented microbes was tracked using high specificity molecular tracking tools explained previously (D'Imperio et al., 2011; D'Imperio et al., 2013). It was observed that bioaugmenting with this product not only significantly improved the overall sCOD degradation in the bioaugmented reactors but also helped stabilize their nitrification activity. The organic stress from the naturally occurring nitrifying microbial community in a treatment plant can be removed by the introduction of non-nitrifying, heterotrophic microbes which can lead to improved nitrification performance. Further the growth of the bioaugmented microbes was strongly correlated with the sCOD removal performance improvement observed in the bioaugmented reactors.

INTRODUCTION

Bioaugmentation technology can be successfully used for optimizing the microbial community responsible for wastewater treatment (D'Imperio et al., 2013). Establishing the link between changes in the overall microbial community due to bioaugmentation and treatment plant performance improvements is the key for establishing the credibility of the bioaugmentation approach. One main criticism that the bioaugmentation technology receives is that comparing the bioaugmented plant performance with its historical data isn't an ideal comparison especially when there is a high degree of variation in the waste composition and concentration. Also, the authors have previously found that it is very difficult to isolate two different trains of a full-scale treatment plant and prevent cross contamination of the non-bioaugmented train. Further, comparing two separate treatment trains at full-scale may not provide the statistical validity of the obtained results. To address such concerns, a battery of twelve flow-through activated sludge reactors was operated in a laboratory using a synthetic wastewater medium. The current study demonstrates one of the very first correlations between the bioaugmentation culture persistence, changes in the overall microbial community due to bioaugmentation and the reactor performance benefits using latest molecular biology tools such as high specificity q-PCR and *16S rRNA* gene metagenomics. The study presented herein used a commercially available microbial product that didn't have any nitrifying ability to bioaugment a battery of lab-scale flow-through activated

sludge reactors. It was observed that bioaugmenting with this product not only improved the overall sCOD degradation in the bioaugmented reactors but also helped stabilize the nitrification activity. This interestingly indicates that the organic stress from the naturally occurring nitrifying microbial community in a stressed treatment plant can be removed using heterotrophic microbes.

METHODS

Draft genomes of the nine bacterial strains in a commercially available bioaugmentation product BioRemove 5100™ were prepared. Strain-specific qPCR primers and probes were designed for each individual microbe & qPCR standard curves were developed for the strains using different sludge-microbe dilutions as previously described (D'Imperio et al., 2011). Twelve 18 L size Eckenfelder type flow-through activated sludge reactors were operated using a complex high strength municipal wastewater medium (composition given below in Table 1) and activated sludge collected from a local municipal wastewater treatment plant. The model of Eckenfelder type reactor used for the study is shown in Figure 1.

Table 1. Synthetic wastewater medium used for the laboratory bioaugmentation study

Macro Nutrients	**Concentration, (mg/L)**
Peptone	384
Meat Extract	264
Yeast Extract	90
Sucrose	200
Urea	192
NH_4Cl	180
K_2HPO_4	89.6
NaCl	22.4
$CaCl_2 \cdot 2H_2O$	12.8
$MgSO_4 \cdot 7H_2O$	6.4
$NaHCO_3$	1000
Micro (trace) Nutrients	**Concentration, (mg/L)**
$FeSO_4 \cdot 7H_2O$	2.24
$ZnSO_4 \cdot 7H_2O$	11.2
$MnSO_4 \cdot H_2O$	6.72
$CoCl_2 \cdot 6H_2O$	1.92
$CuSO_4 \cdot 5H_2O$	2.00
$NaMoO_4 \cdot 2H_2O$	1.92

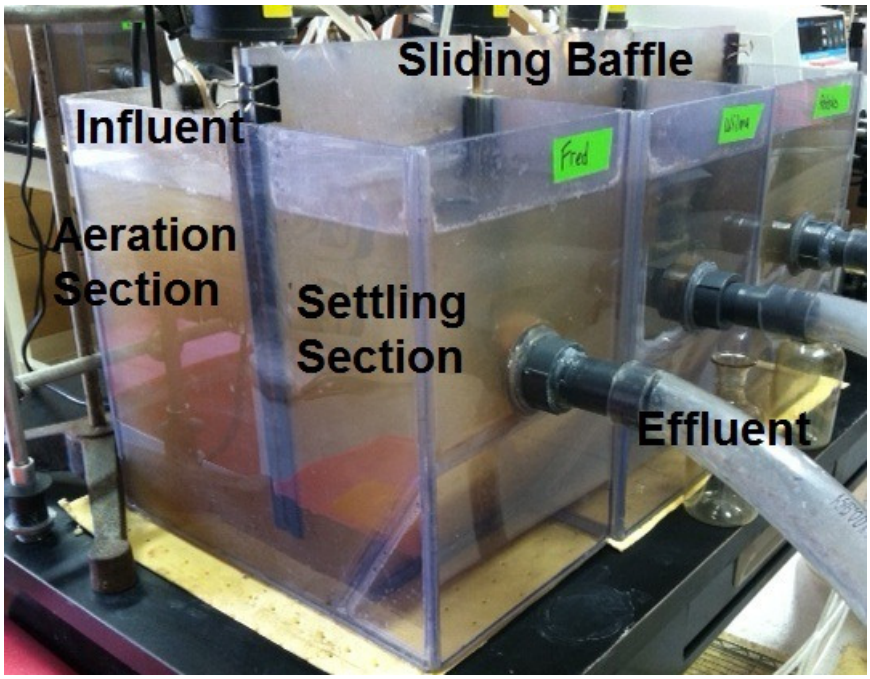

Figure 1. Eckenfelder type flow-through reactors used for the laboratory bioaugmentation study

The reactors were started with very low initial MLSS concentration (<0.25 mg/L) to cause artificial stress conditions. The reactors were equally divided into two sets of six and operated at > 0.2 F/M loadings. One set of the reactors received 15 mg/(Linflow-day) of BioRemove 4100 bioaugmentation product daily, resulting in approximately 10^2 cells/mL of each bacterial culture in the reactor volume whereas the other set wasn't bioaugmented. The reactor sets were placed apart from each other to avoid cross contamination of the bioaugmented microbes into the non-bioaugmented reactors. Periodic samples were taken from the reactors and analyzed for MLVSS, soluble COD, NH_3-N, NO_3-N and NO_2-N using procedures listed in the Standard Methods (APHA et al., 1998). Concentrations of all the bioaugmented strains were tracked using strain-specific qPCR protocols described previously (D'Imperio et al., 2011). Multiple samples from each reactor were analyzed for metagenomic analysis using *16S rRNA* gene sequences and Illumina pyrosequencing at different time points.

RESULTS

The bioaugmentation product BioRemove 5100™ used in the present study didn't have any strains with ammonia or nitrite oxidation capabilities (confirmed separately by ammonia oxidation study as well as metagenomic analysis of the product, data not shown herein) but surprisingly better nitrification was observed in the treated reactors following the daily bioaugmentation. The bioaugmented reactors maintained a stable nitrification performance indicated by minimum ammonia and nitrite accumulation as well as stable nitrate production throughout the treatment period (see Figure 2, 3 and 4).

The non-bioaugmented reactors accumulated significantly higher amounts of ammonia during the first 28 days of the experiment indicating poor ammonia oxidation activity as shown in Figure 2. Following the first 28 days of operation, the untreated reactors started oxidizing ammonia but the oxidized ammonia accumulated in the form of nitrite in the these reactors indicating poor nitrite oxidation efficiency throughout the experiment as shown in Figure 3 and 4. Metagenomic analysis of the microbial communities from the treated and the untreated reactors indicated that bioaugmentation with non-nitrifying microbes didn't significantly change the nitrifying community (Figure 5) but may have favored the activity of the nitrifying community in the reactors. This effect may be a result of reduced overall organic stress in the treated reactors enabling the pre-existing nitrifying community to perform better leading to better nitrification as compared to the untreated reactors.

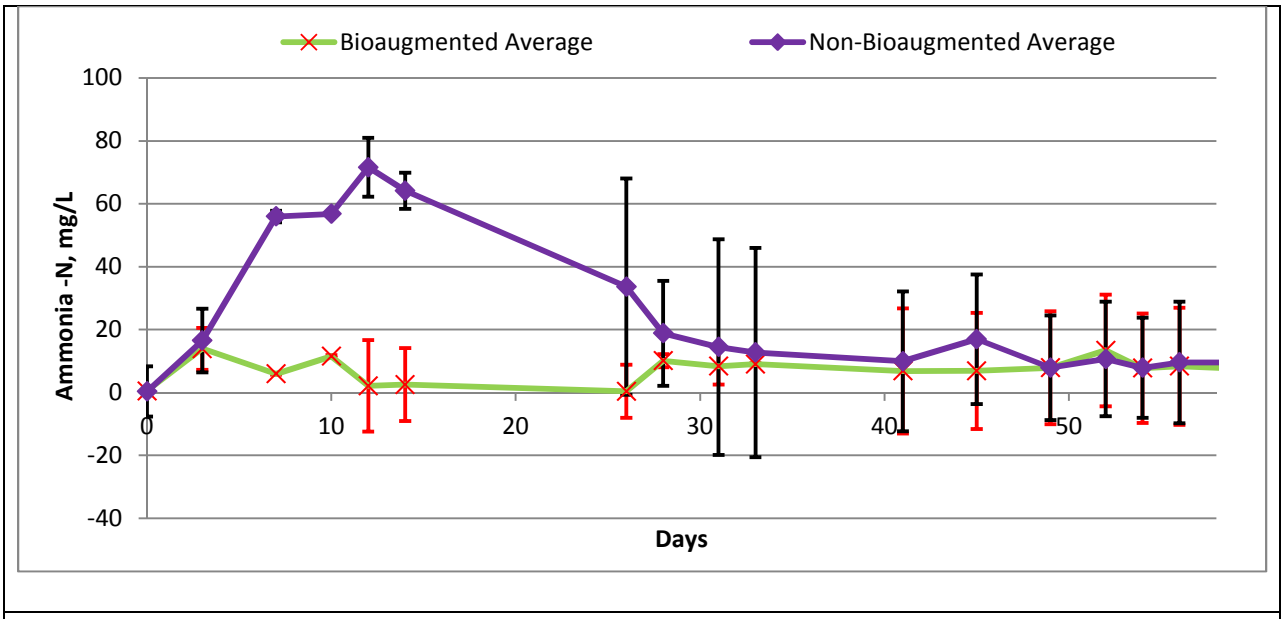

Figure 2. Bioaugmented digesters showed no ammonia accumulation indicating better ammonia oxidizing function. Error bars indicate standard deviation among six replicates

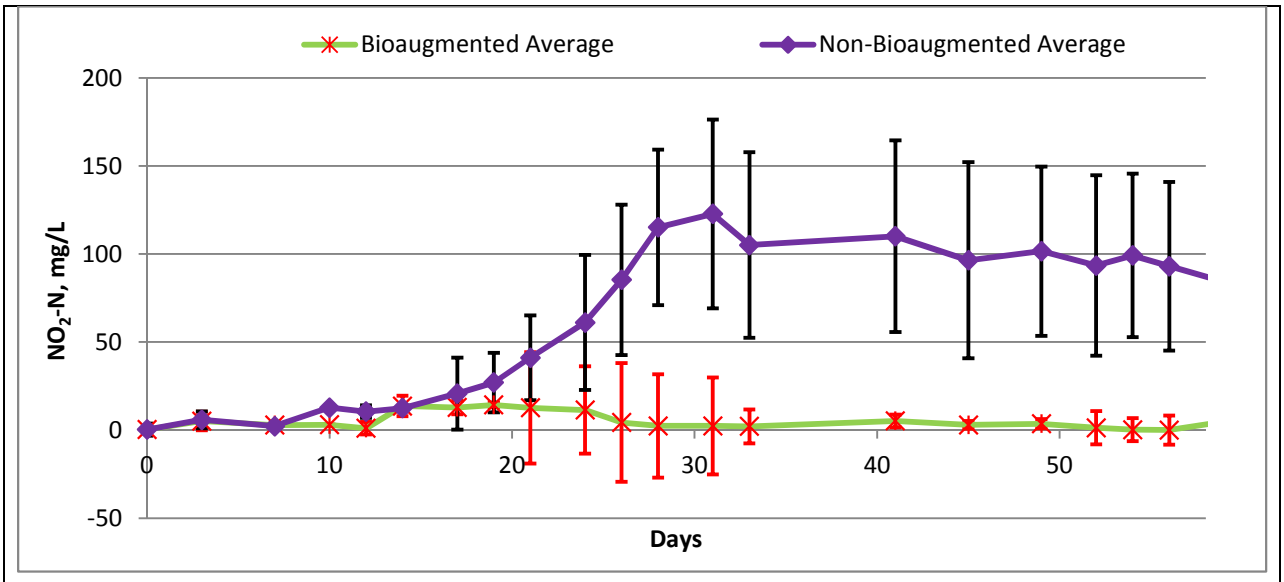

Figure 3. Bioaugmented digesters showed consistently lower nitrite accumulation. Error bars indicate standard deviation among six replicates

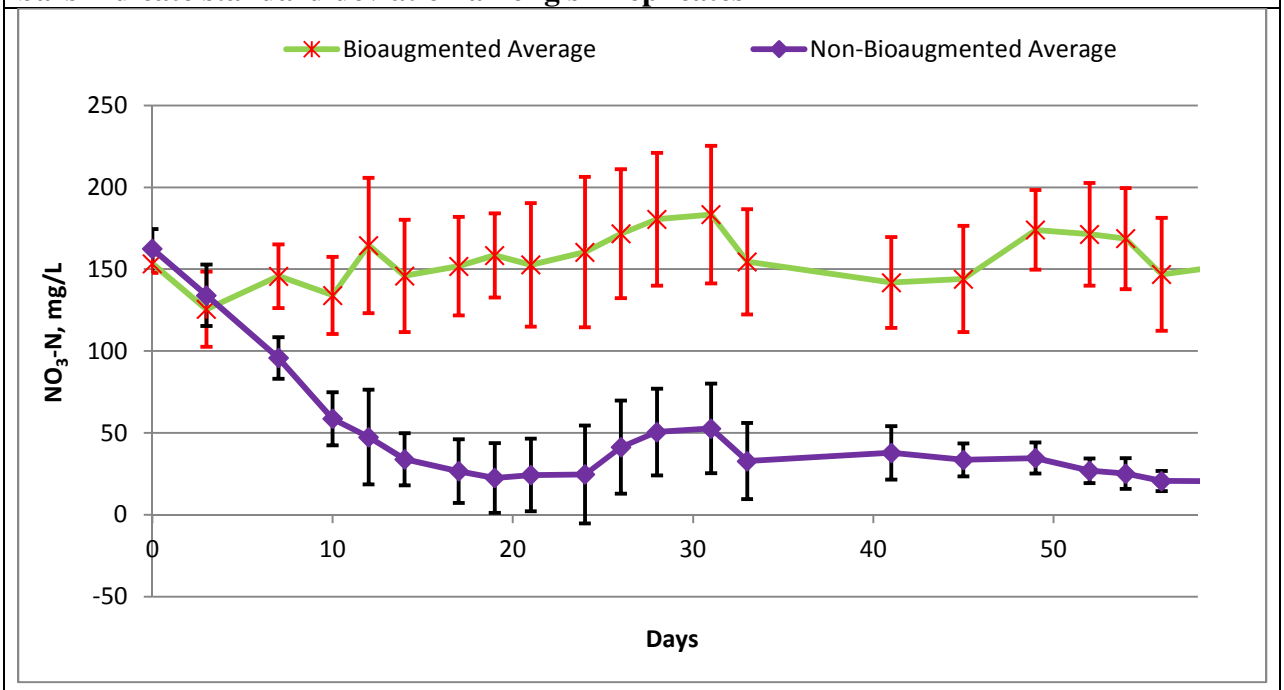

Figure 4. Bioaugmented digesters showed consistently higher nitrite production indicating better ammonia and nitrite oxidation performance. Error bars indicate standard deviation among six replicates

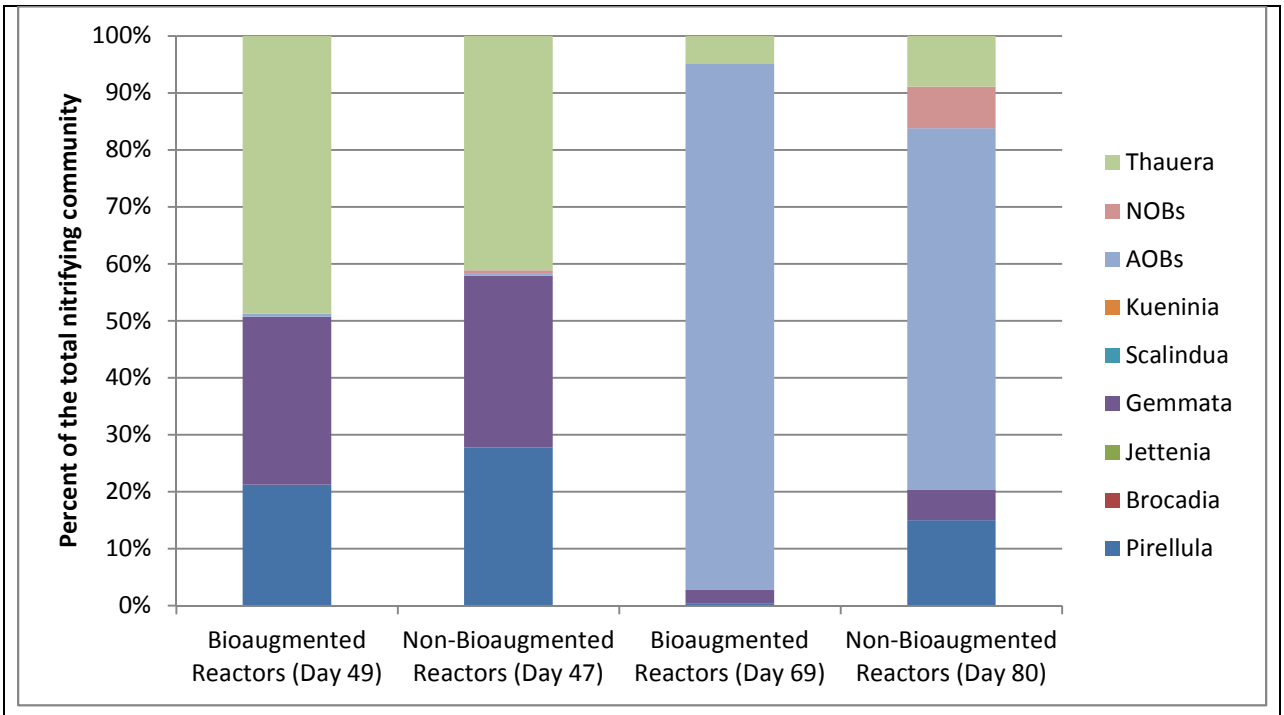

Figure 5. Comparative analysis on nitrifying bacterial community due to bioaugmentation Values presented are averages of two values

Figure 6 shows the effluent sCOD concentrations of the bioaugmented and non-bioaugmented reactors. The bioaugmented reactors reached COD degradation efficiency of more than 90% immediately within first 24 hrs upon the startup, whereas the non- bioaugmented reactors did not achieve > 90% COD removal efficiency until the end of the experiment. During the entire duration of the experiment, the bioaugmented reactors performed better than the non-bioaugmented reactors in terms of better sCOD removal. Figure 7 indicates the concentration of the bioaugmented strains measured using high specificity qPCR technique. Out of the nine strains used for bioaugmentation, strain 3067 and 3091 had significantly higher growth (2 to 7 log increase) upon bioaugmentation.

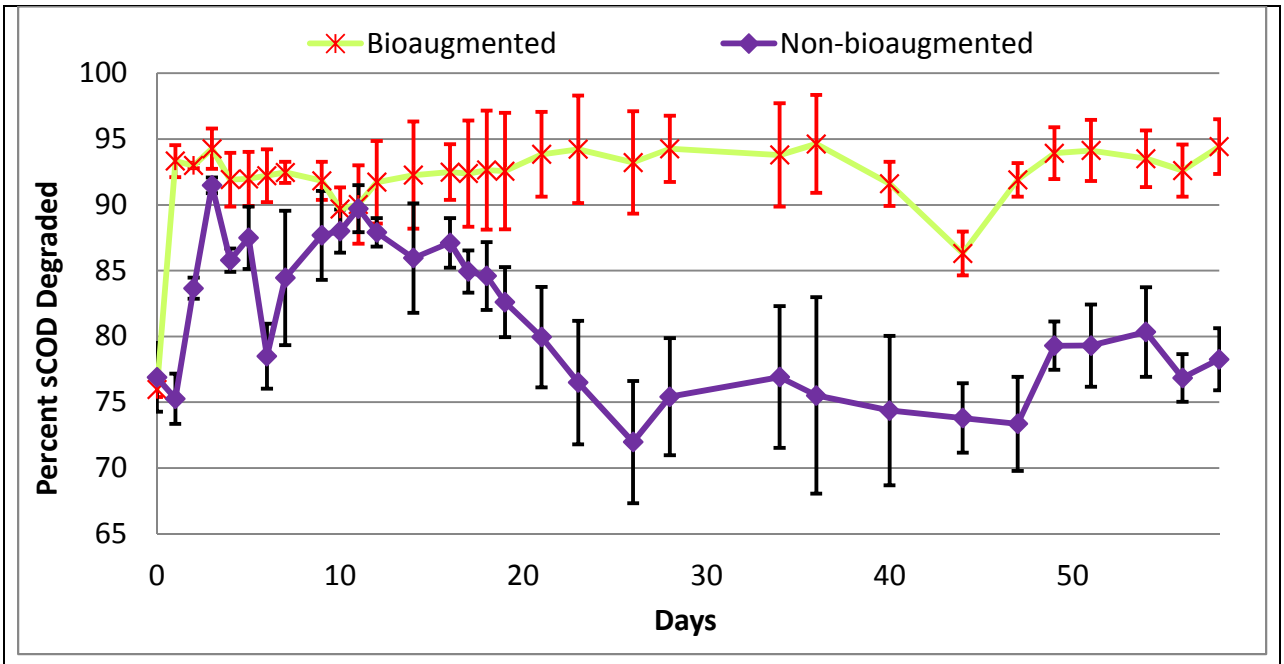

Figure 6. Bioaugmented digesters showed consistently better and stable performance as compared to the non-bioaugmented digesters. Error bars indicate standard deviation among six replicates

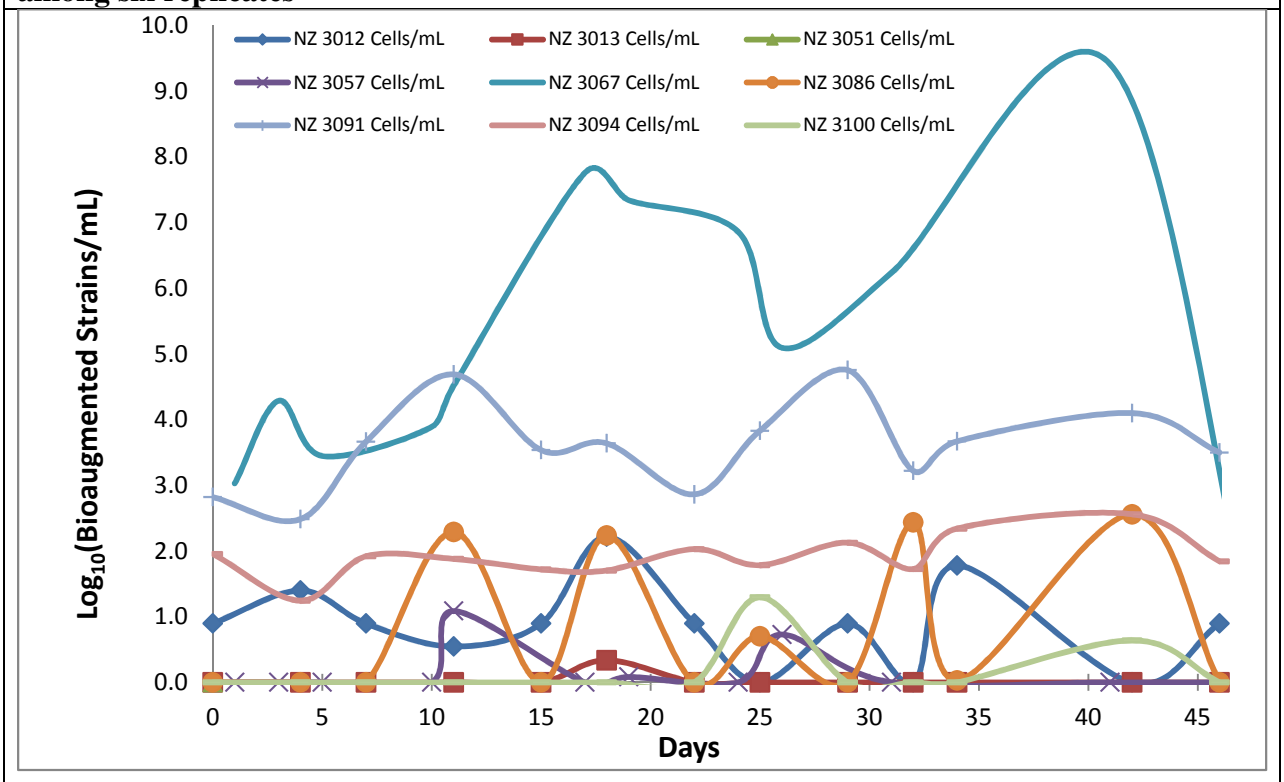

Figure 7. Actual concentration of the bioaugmented strains in the mixed liquor measured by qPCR. Concentrations above the dosing line indicate growth

Figure 8 shows the correlation between the measured strain concentration of the bioaugmented cells and the COD removal performance improvement observed in the treated reactors over the untreated reactors. It is evident from the graph that there is a strong correlation between the persistence of the bioaugmented strains and the bioaugmented reactor performance improvement. The current results of strong correlation between the bioaugmented strain grown and reactor performance improvement complement the previously published report based on a field study (D'Imperio et al., 2013) highlighting the effectiveness of the bioaugmentation approach.

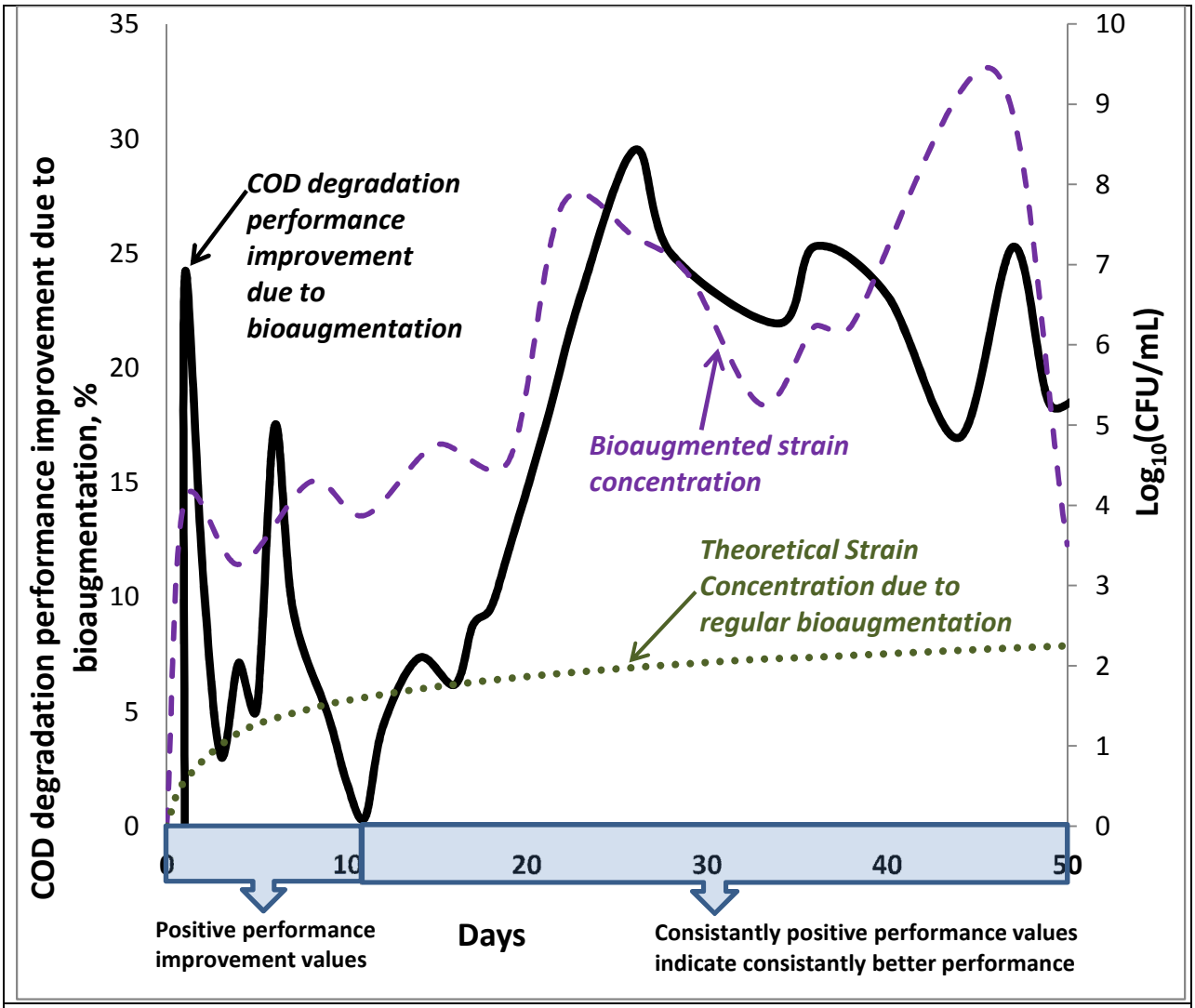

Figure 8. Theoretical total strain concentration (in case of no growth) vs measured total strain concentration and COD removal performance improvement calculated as percent extra COD removed by treated reactors as compared to the untreated reactors

CONCLUSIONS

Bioaugmenting with non-nitrifying microbial strains can help reduce overall stress on the preexisting nitrifying community in a wastewater treatment environment and hence bioaugmentation can lead to improved nitrification efficiency in the stressed reactors.

Current lab study shows that it is possible to quantify the growth of bioaugmented strains in a mixed microbial community environment using advanced, strain-specific molecular tracking tools. These tools can help correlate the growth of the bioaugmented strains with the performance improvement observed in the bioaugmented reactors.

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