Skermania piniformis Foaming Incident in a Biological Nutrient Removal Plant in Southern California

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Executive Summary

Foaming bacteria, *Skermania*-like spp., were investigated over 54 weeks from 2016 to 2017. *Skermania*-like spp. was identified as the cause of foaming at the study site via DNA sequencing. The study site fully nitrified and partially denitrify wastewater. *Skermania*-like abundance ranged from 2.49×10^{8} to 1.32×10^{13} cells/L and were quantified using the quantitative Polymerase Chain Reaction (qPCR) method. The ratio of COD to total nitrogen in primary effluent ratio was first observed to have an inverse relationship with the *Skermania*-like cell abundance, but no significance was reported. The Pearson correlation coefficient (r) and degree of significance (P) were tested between *Skermania*-like abundance and several physicochemical factors and plant operational parameters. The data indicated an inverse relationship between *Skermania*-like population and temperature (r = -0.31; P < 0.05) as well as *Skermania*-like population and total nitrogen in influent (r = -0.31; P < 0.05). This implied that *Skermania*-like outcompeted other bacteria for substrate during cool weather. Since the plant tried to fully remove nitrogen in wastewater, all plant parameters were adjusted for complete nitrification and denitrification. *Skermania*-like spp. were affected by low nitrogen concentrations and benefitted more from the substrate than other nitrifying and denitrifying bacteria.

Project Objectives

The ultimate goals of this project were to identify foaming bacteria in activated sludge and to determine causes initiated and promoted foaming incidents at this study site using Pearson Correlation Coefficients and Degree of Significant

Project Approach

Study Site: 54 weekly samples were collected from a Water Reclamation Plant in Southern California with a capacity of 25 million gallons per day (MGD). This plant completely nitrified and partially denitrified wastewater using a plug flow step feed configuration. There was a total of six samples taken each week. The samples used in this project were from anoxic tank stage 1.

DNA extraction: Nucleic acids were extracted using bead-beating protocol with phenol/chloroform followed by chloroform purification (Yu and Mohn, 1999; Asvapathanagul et. al, 2012), which was fully described in Asvapathanagul et. al, 2012 (see Figure 1).

DNA quantification by qPCR: The annealing temperature is 59°C. Sybr Green assay was employed and the master mixture preparation followed SYBR Green Maxima (Thermo Fisher Scientific)

Statistical calculation. To test the correlation between two variables, the Pearson correlation coefficient (*r*) was used, and when significance (*p)* was found, the *p* value accompanies the *r* value. The statistical significance (*p*) was calculated using the T-distribution function (TDIST) of Microsoft excel for both tails. For the statistical analysis, the sample number of 54 was used for the period.

Project Outcomes

- Primary effluent characteristics were displayed in Table 1a,and Pearson correlation coefficient (*r*) values between *Skermania*-like spp. and other parameters was showed in Table 1b.
- The lower the temperature, the higher the *Skermania*-like cell abundance, especially when temperature lagged one week behind (Figure 2).
- Lagged temperature statistically demonstrated the highest inverse Pearson correlation coefficient with a value of -0.31 ($P < 0.05$). This indicated temperature was a factor that promoted *Skermania*-like's growth (Figure 2).
- The COD to total nitrogen ratio (C/N) in primary effluent showed a weak correlation (r = 0.16, $P > 0.05$). However, the negative trend of C/N ratio and *Skermania*-like spp. cell population was observed in Figure 3.
- A strong inverse relationship between *Skermania*-like spp. and total nitrogen in primary effluent was $r = -0.31$, $P < 0.05$). This implied that insufficient amounts of nitrogen substrate induced excessive growth of *Skermania*-like spp. foaming bacteria (Figure 4).

Conclusions

Temperature and available total nitrogen in primary effluent were found to be significant co-factors (r = -0.32 and -0.31, respectively) that promote the growth of *Skermania* spp. at this study site.

Future Work

Remaining samples include those from aeration 1, anoxic 2-3, and aeration 2-3 can be calculated the *Skermania* spp. growth rate.

Appendices (if any)

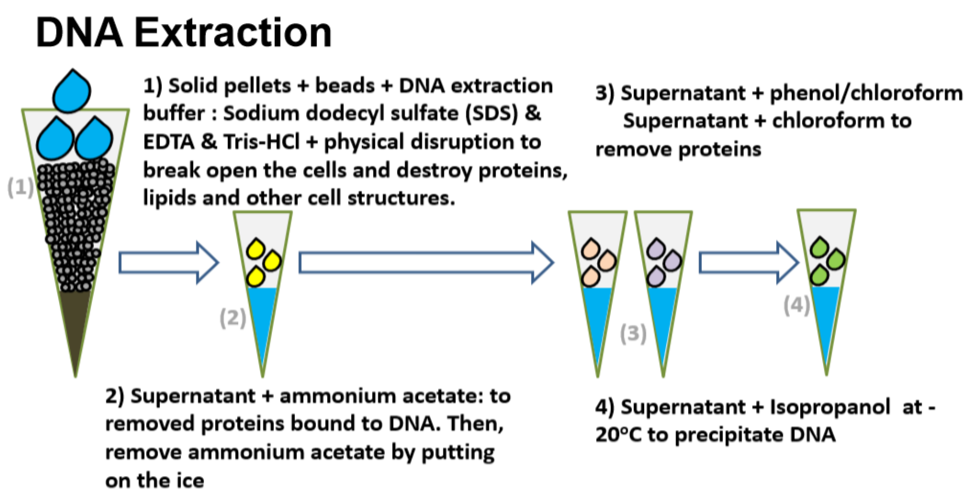

Figure 1 DNA extraction protocol (Yu and Mohn, 1999; Asvapathanagul et. al, 2012)

Table 1 Primary effluent characteristic (a, left), Pearson correlation coefficient between *Skermania*-like spp. and other parameters (b, right)

Parameter	Primary Effluent
Flow Rate (MGD)	11.56 - 21.18
Temperature (°C)	1.92
BOD (mg/L)	161.45 - 301.95
COD (mg/L)	362.47 - 563.63
NH_4^+ (mg/L)	32.96 - 43.23
TSS (mg/L)	75.28 - 181.29
VSS (mg/L)	78.43 - 83.13

Parameter	r
COD, Primary	0.03
Nitrogen, Primary	-0.31*
COD/Nitrogen	0.16
COD 4 Average**	-0.09
COD 7 Average***	-0.04
Nitrogen/COD	-0.03
COD/Nitrogen	0.02
COD/Nitrogen Lag	-0.01
Temperature	-0.27
Temperature Lag	-0.32*

* Indicating $P < 0.05$ as a significant relationship; ** indicating 4-day average COD; *** indicating 7-day average COD.

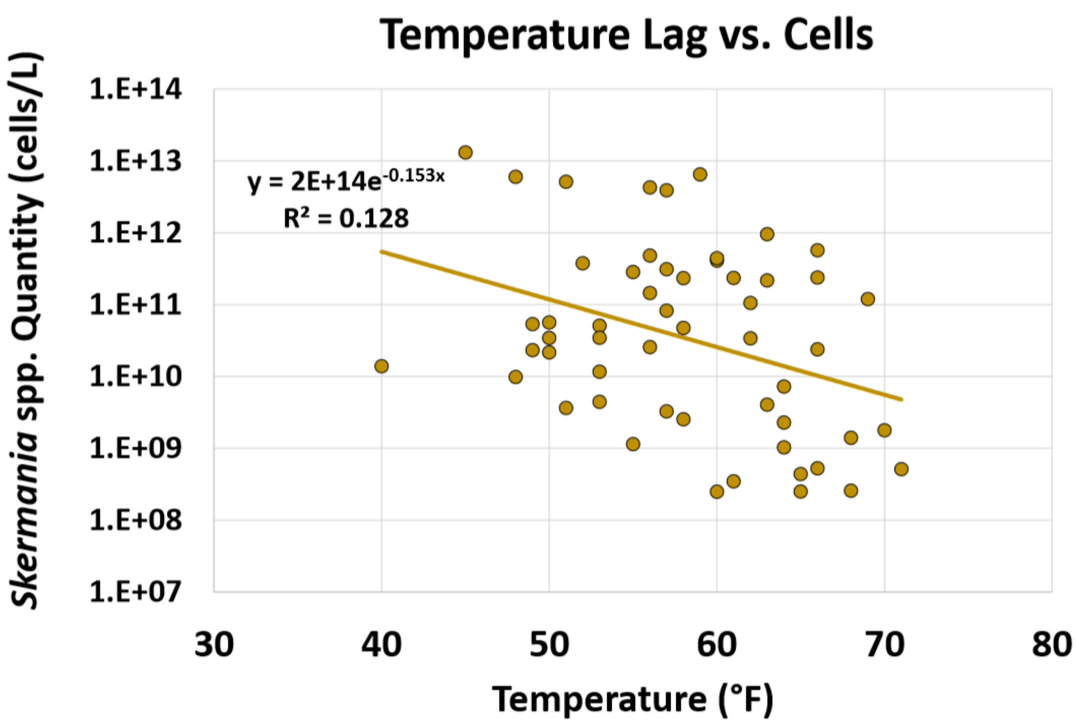

Figure 2 Relationship between temperature lagged and *Skermania*-like spp. cell abundance

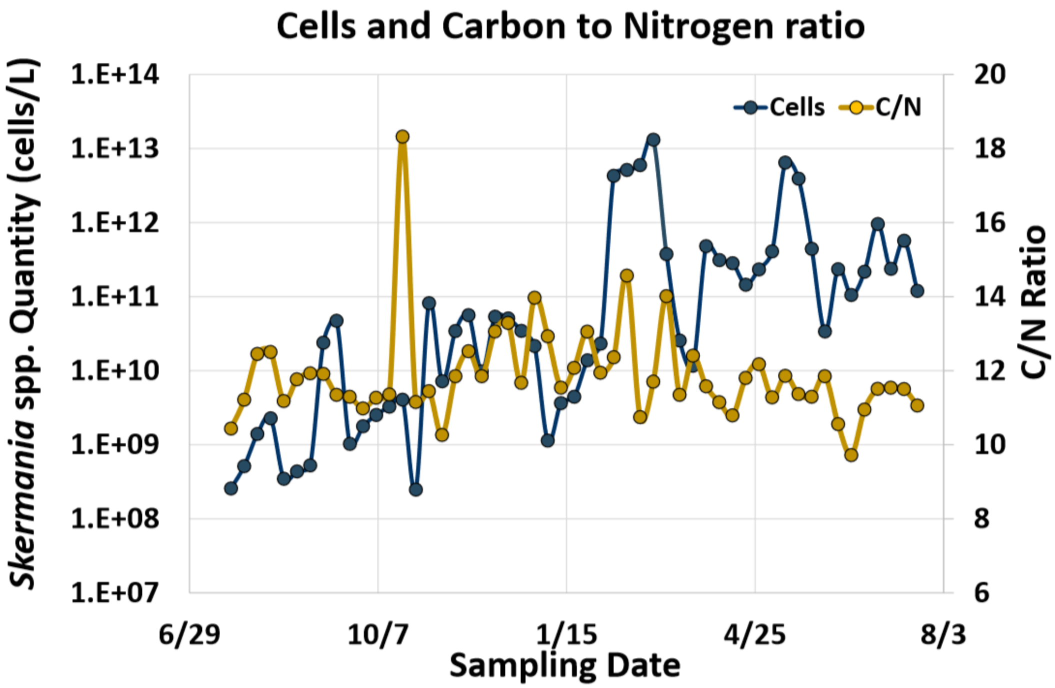

Figure 3 Relationship between carbon to nitrogen ratio in primary effluent and *Skermania*-like spp. cell abundance over the project campaign.

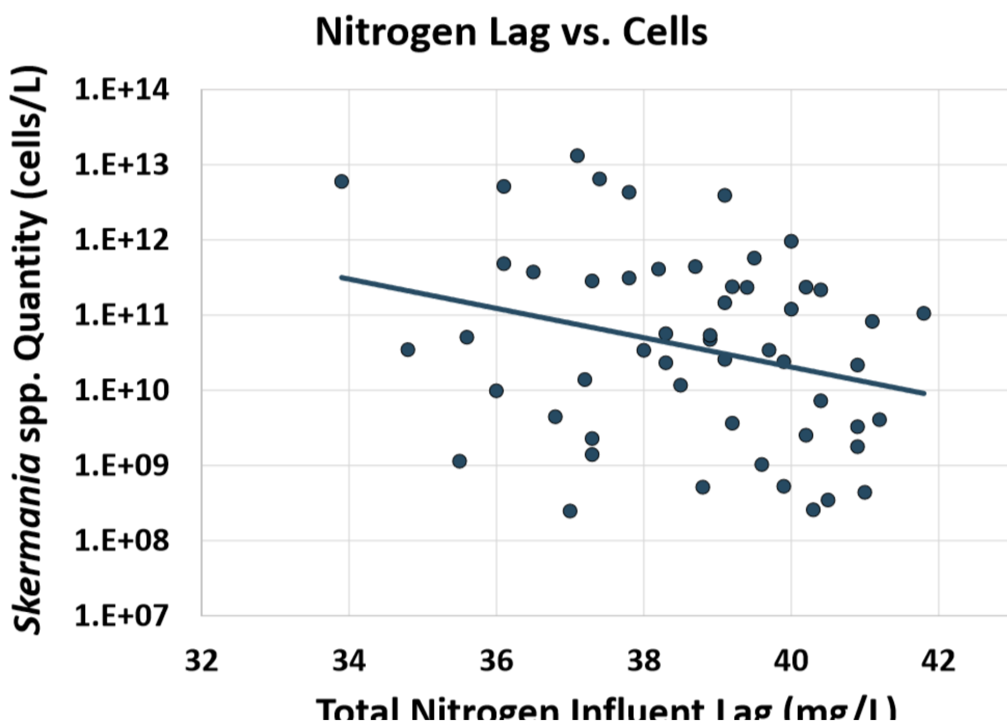

Figure 4 Influent total nitrogen lagged and *Skermania*-like spp. cell abundance

Reference

Asvapathanagul, P., Huang, Z.H., Gedalanga, P.B., Baylor, A., Olson, B.H. (2012) Interaction of operational and physicochemical factors leading to Gordonia amarae-like foaming in an incompletely nitrifying activated sludge plant. Appl Environ Microbiol 78(23), 8165-8175.

Yu, Z.T., Mohn, W.W. (1999) Killing two birds with one stone: simultaneous extraction of DNA and RNA from activated sludge biomass. Can J Microbiol 45(3), 269272