Community Diversity and Metabolic Potential of Marine Aquaculture Trickling Filter Biofilms

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Introduction

A cogent system of theory on the importance of community diversity on community metabolism and function is an important foundation to leverage our understanding of microbial ecosystem structure and function (Prosser, 2007), and is particularly important for understanding biofilm communities because of their complex spatial microenvironments, which mirror macroscopic ecological landscapes (Battin, 2007). Engineered systems have been cited as an integral subject for studies in theoretical ecology because a strong theoretical system can improve predictive power and process stability in engineered environments (Briones and Raskin, 2003). An understanding of how conserved microbial communities are across physical and chemical gradients in the environment is important in industrial systems because robust communities may increase process stability. Re-circulating marine aquiculture systems are at a high risk of upset from pathogen introduction or failure of biological treatment to effectively facilitate nutrient removal. However, the conservation of microbial communities under different physical and chemical conditions has not been fully explored. Previous studies of microbial diversity of similar systems have identified sulfur oxidizers from the Gamma-proteobacteria and Beta- and Alphaproteobacteria as dominant groups (Cytryn et al., 2003, Cytryn et al., 2005). Ammonia oxidizers (*Nitrosomonas*) and nitrite oxidizers (*Nitrospira*) as well as Planctomycetales performing anammox have also been detected (Tal, 2003, Tal et al., 2006). The goal of this work was to compare the diversity of microbial communities grown under different temperature and nutrient conditions and to compare the metabolic potential of recently-assembled and mature biofilms under these different conditions.

Materials and Methods

Sampling

Samples were collected from the Marine Resources Center (MRC) at the Marine Biological Laboratory in Woods Hole, MA. The MRC is a re-circulating marine aquaculture facility with seven independent re-circulating loops that are biologically treated with trickling filters (the surface area of the filters is approximately 10,000 square feet). Biomass and bulk water samples were collected aseptically and immediately processed or stored at -80 ºC.

DNA Extraction, PCR, Cloning, and Sequencing

DNA was extracted using a standard phenol-chloroform method (Sambrook and Russell, 1994) and purity and DNA yield were determined spectrophotometrically (Nanodrop ND-1000). The primers 519F (CAGCACGCCGCGGTAATFWC) and 1392R (ACGGGCGGTGTGTRC) were used to target bacteria and archaea. A Promega kit was used for PCR reactions. 30 cycles of denaturation (95 C, 30 sec), annealing (46 C, 30 sec) and extension (72 C, 1.5 min) were used for the PCR reaction. 1% agarose gels were used to confirm the presence and quality of PCR product. Cloning was conducted using the Invitrogen TOPO TA cloning kit and sequencing was conducted at the Marine Biological Laboratory Sequencing Core Facility.

Respirometry

Oxygen consumption rates were measured using Unisense respirometer chambers and dissolved oxygen (DO) probes (Aarhus, Denmark). DO probes were calibrated with a 2 point (zero and saturation oxygen) calibration at the start of every experiment and were also confirmed during experiments. The Branford assay was used to determine the protein content of biofilms

(Bradford, 1976). Biofilms were sonicated for 10 minutes at maximum power to ensure removal of proteins from biofilms before the Bradford assay.

Water Quality Analysis
Temperature was measured online by staff at the MRC. Ammonia and nitrate were measured in triplicate in the analytical chemistry laboratories at the Ecosystem Center at the Marine Biological Laboratory.

Analysis Software
Alignment was conducted using ARB (http://www.arb-home.de/) and the GreenGenes alignment service (greengenes.lbl.gov). Additional analysis was conducted using DOTUR (http://www.plantpath.wisc.edu/fac/joh/dotur.html) and S-Libshuff (http://www.plantpath.wisc.edu/fac/joh/S-libshuff.html).

Results and Discussion

Sampling Site Water Quality

The Marine Resource Center has eight parallel loops for re-circulating marine aquaculture applications. Different applications require different physical and chemical water quality characteristics, so the loops are exposed to myriad water chemistries and temperatures. Two of the eight loops (5 and 7) were selected for this study because of a significant temperature difference between the two (27 and 16 ºC, respectively). As illustrated in Table 1, ammonia levels are similar for the two loops, although the nitrate levels are over three times higher in loop 5. In addition, the total organic carbon of the water passing through the filters is very similar between the two loops.

Loop	NH_3, µM	NO_3, µM	Total Organic Carbon, µM as CO_2	Temp, ºC
5	1.25	15	139.4	27
7	1.21	4.37	132.4	16

Table 1: Water Quality Parameters of Trickling Filters

Phylogenetic Diversity and Community Characteristics

a. Rarefaction

Bacterial and archaeal 16S rRNA clone libraries were constructed for biofilm communities growing on plastic "bioballs" within trickling filters 5 and 7. Alignments were made using ARB software and a neighbor-joining matrix was then exported to the software DOTUR for further analysis. Rarefaction curves were made within DOTUR for both libraries compiled (Figure 1) and each library individually (Figure 2). Figure 1 illustrates that the rarefaction curve does not achieve a plateau until a sequence similarity of <0.9 is used to classify an operational taxonomic unit. Figure 2 further demonstrates that the system is under-sampled by considering the biofilm

community from each loop separately and defining the OTU at the species level (97% sequence similarity).

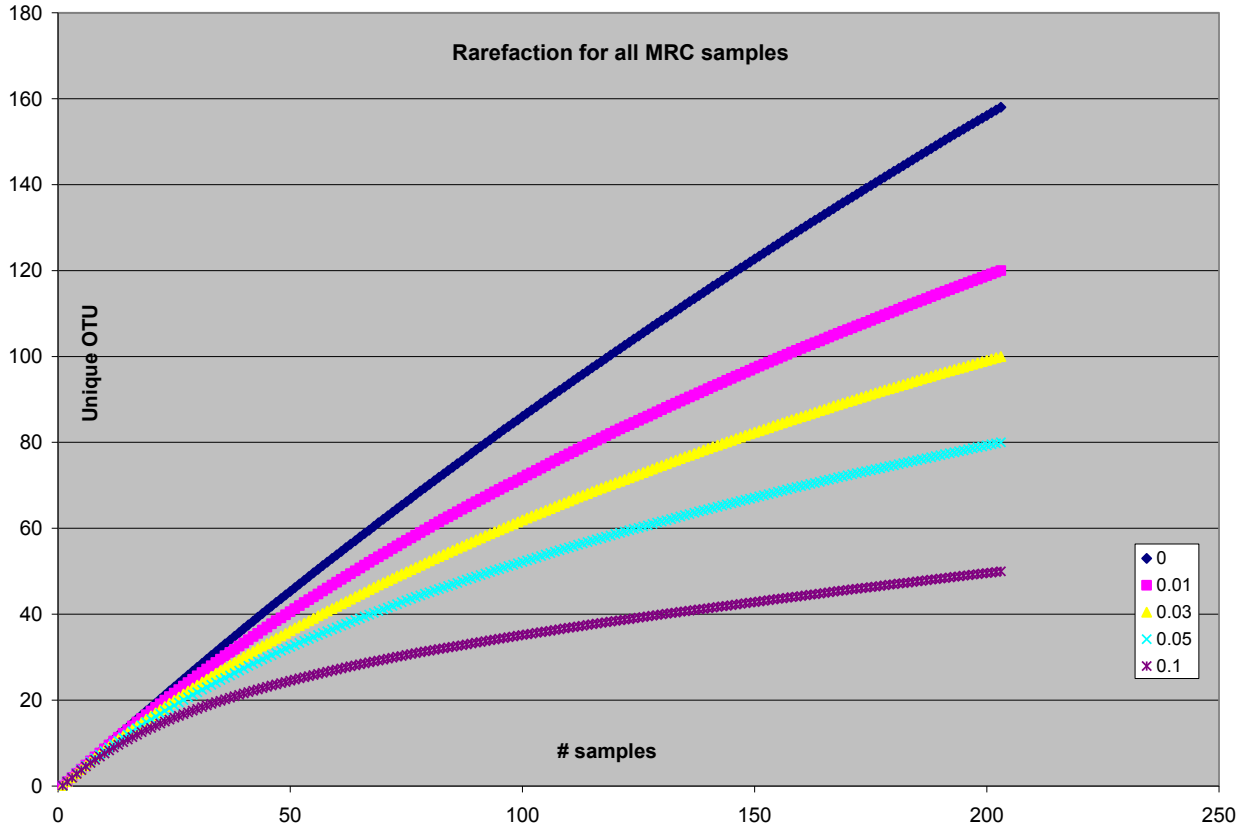


Figure 1: Rarefaction Curve for Combined Libraries

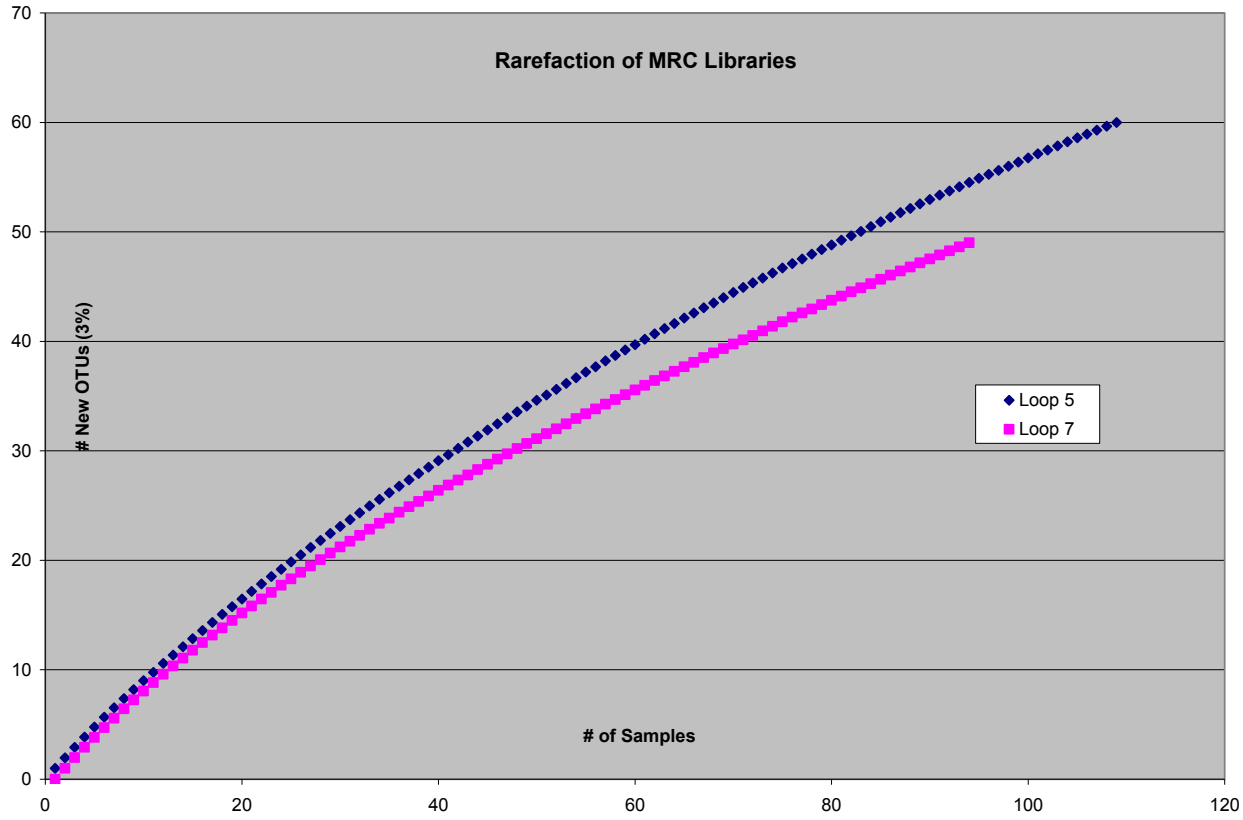


Figure 2: Rarefaction Curve for Individual Libraries

b. Species richness

The species richness of the microbial communities from the two loops was estimated using a Chao 1 estimator. Species richness is a measure of the number of species in a system. The Chao 1 estimator is a useful tool for inferring the actual species richness of a community from a finite number of samples (Chao, 1984). The results of the Chao 1 estimator of species richness suggest that the species richness of the communities in loops 5 and 7 are not significantly different (Figure 3). This means that both systems have a similar number of species present in the community, which may have implications for the metabolic diversity and metabolic activity potential of the community.

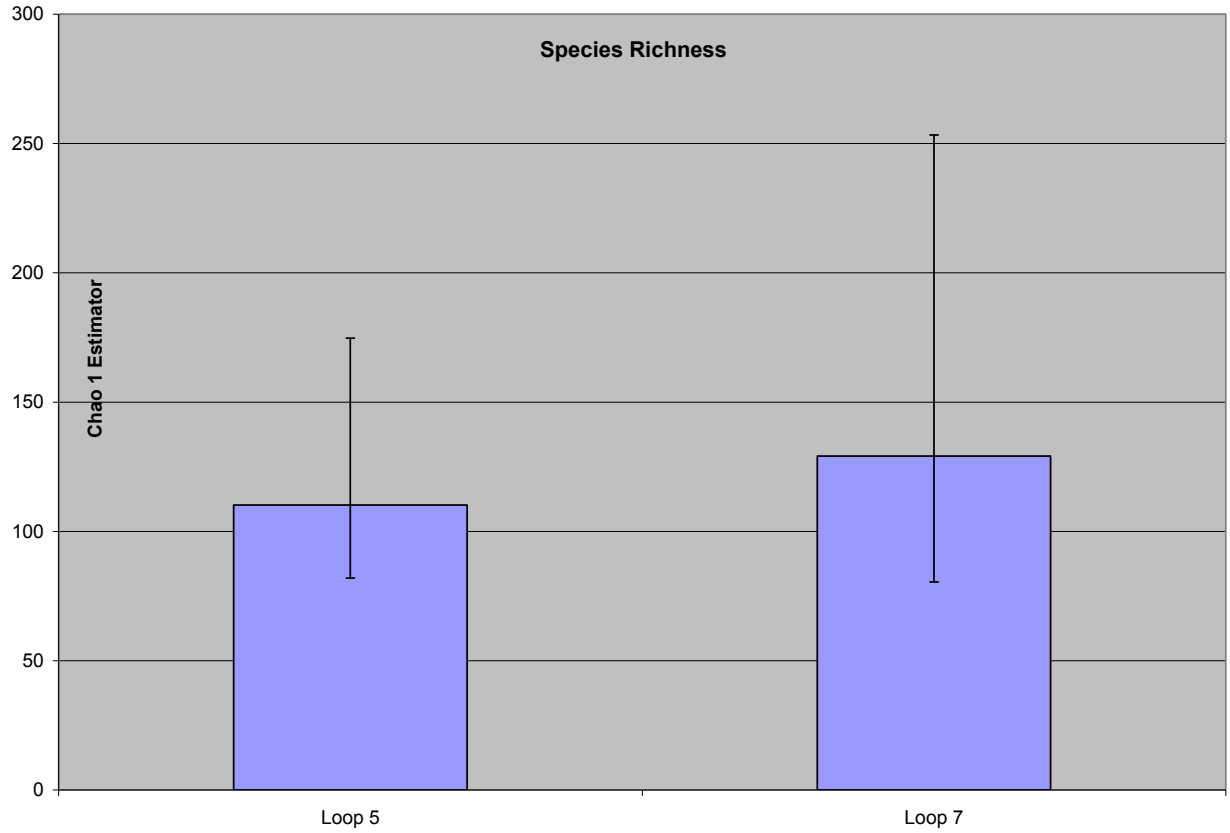


Figure 3: Species Richness of Communities

c. Species diversity

The Shannon-Weaver Index of Diversity was used to compare the species diversity of the two biofilm communities (Figure 4). This analysis takes into account both the number of species present in a community and the evenness of their populations. Therefore, while any two communities may have the same species richness, they may have different Shannon-Weaver Indices of Diversity if one community has one or two dominant species and the other community has fairly even population sizes. The results from Figure 4 show that while Loop 5 has a slightly higher Shannon-Weaver Index than Loop 7, the error bars included for a 95% confidence range are large enough that the difference in the mean value is not statistically significant.

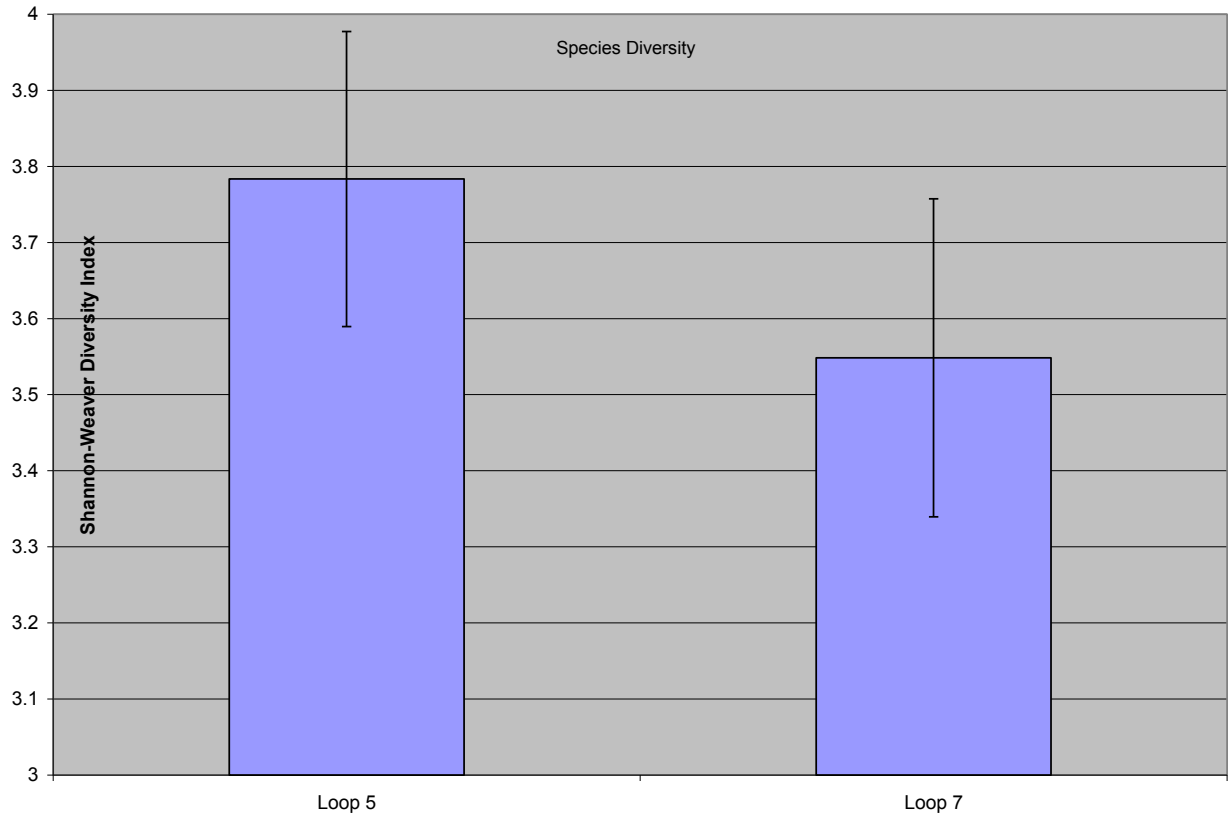
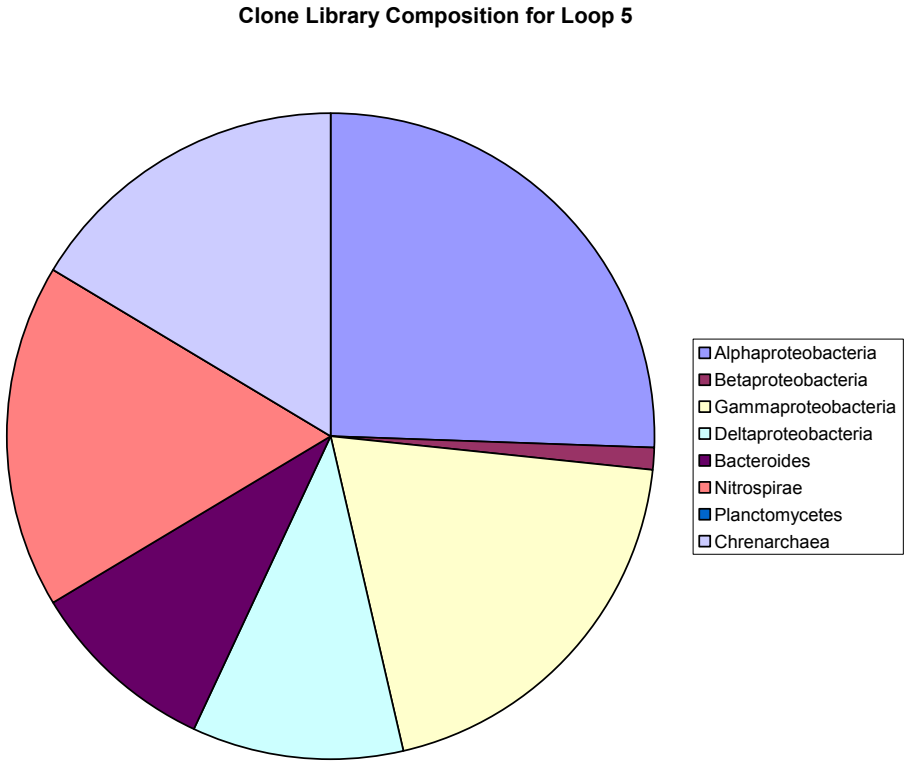
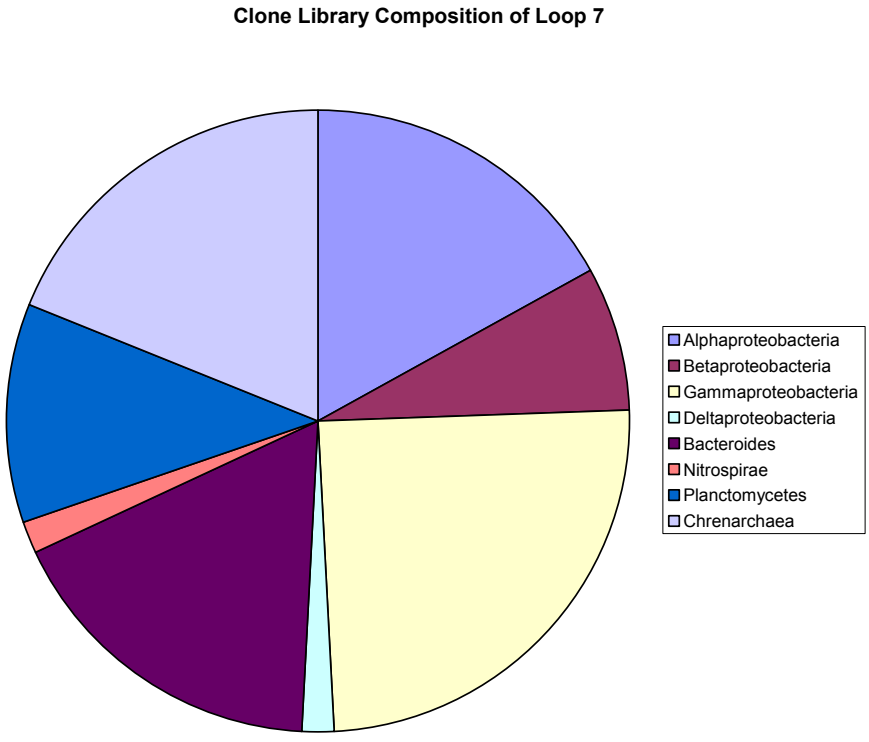


Figure 4: Species Diversity of Communities

d. Community Composition

The community composition was determined by sequence analysis and dividing clones from the library into major subgroups (Figures 5a and 5b). By visual inspection of the highly-represented subgroups in Figures 5a and 5b, it appeared than the community in loop 5 was dominated by Alpha- and Betaproteobacteria, Nitrospirae, and Chrenarchaea. Loop 7, alternatively, was dominated by Alpha- and Gammaproteobacteria, Bacteroides, Planktomycetes, and Chrenarchaeota. Visual inspection of this sort may lead us to suppose that the communities are in fact 'different', but to more objectively answer this question, S-Libshuff was applied to answer the question of whether two communities are truly different. The S-Libshuff analysis returned p values >0.05, indicating that the communities were in fact not significantly different (Table 2). Keeping in mind that the system is under-sampled, this analysis does not exclude the possibility that the communities are in fact different, but that the data set is too small to demonstrate this with the level of confidence required for a rigorous statistical analysis.





Figures 5a and 5b: Community Composition as determined by clone library representation

	Y	
X	1	2
1	0.0000	0.2027
2	0.0927	0.0000

Table 2: S-libshuff Analysis of Community Difference

Metabolic Potential

The metabolic potential portion of this study explored whether biofilms grown under slightly different conditions have different metabolic potential. Also, the study compared the metabolic ability of "young" biofilms that had grown on an introduced substratum material for one week with biofilms that had been in the system for over one year. The metabolic experiments were performed in a respirometer and metabolic activity was quantifies as the initial rate of oxygen consumption after addition of a pulse of 1 mM of an electron donor (NH_4^+ or glucose). The rates of consumption were normalized by the protein content of the biofilm (the protein content was determined after the metabolic experiments with the Bradford assay method).

a. Ammonium addition

The addition of ammonium to mature biofilms from loops 5 and 7 resulted in oxygen consumption rates that were not statistically different from each other (Figure 6). This indicates that neither loop selects more strongly for very active nitrifiers. Interestingly, the one-week old biofilms also demonstrated oxygen demand when supplemented with ammonium, indicating that nitrifiers have already colonized these surfaces. The oxygen consumption of the one-week old biofilm in loop 5 has an oxygen consumption rate equivalent to the developed biofilm, indicating that nitrifier communities can assemble and achieve a high metabolic activity very quickly. The one-week old biofilm in loop 7 was not as active as the mature biofilm, but still was measurably active. One possible reason that colonization was faster in loop 5 was that the temperature of the water in loop 5 was higher than in loop 7, which may play an important role in speeding up colonization kinetics.

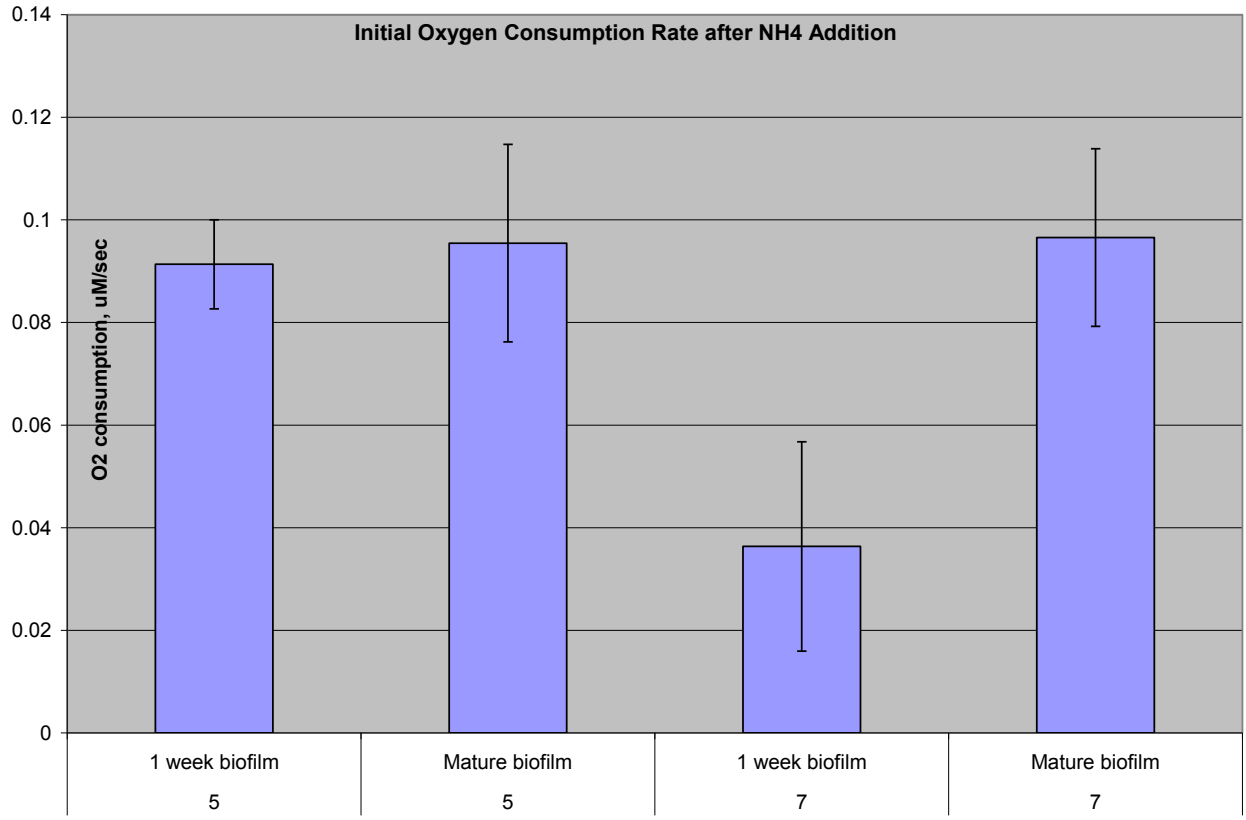


Figure 6: NH_4 Metabolism by Biofilms

a. Glucose addition

Mature biofilms from loops 5 and 7 had equivalent rates of oxygen consumption in the presence of glucose (Figure 7). This finding agrees with the similar rates of oxygen consumption after ammonium addition, and indicates that both biofilms are equally well-suited to heterotrophic metabolism. This also agrees with the water quality data that indicates that the amount of organic carbon in the two systems is very similar. Interestingly, the one-week old biofilms had higher oxygen consumption rates when exposed to glucose than the mature biofilms that were growing in the same loop. A possible explanation of this is that initial colonizers are able to use abundant nutrients more quickly and therefore have a kinetic advantage during nutrient acquisition. Over time, however, the biofilm may become nutrient-depleted. Nutrient depletion will select for organisms that can utilize low level of nutrients but are not kinetically competitive at high concentrations of electron donor. These organisms would then appear to have a lower rate of initial oxygen consumption, though they may be able to more effectively utilize the electron donor to a lower concentration than the early colonizers.

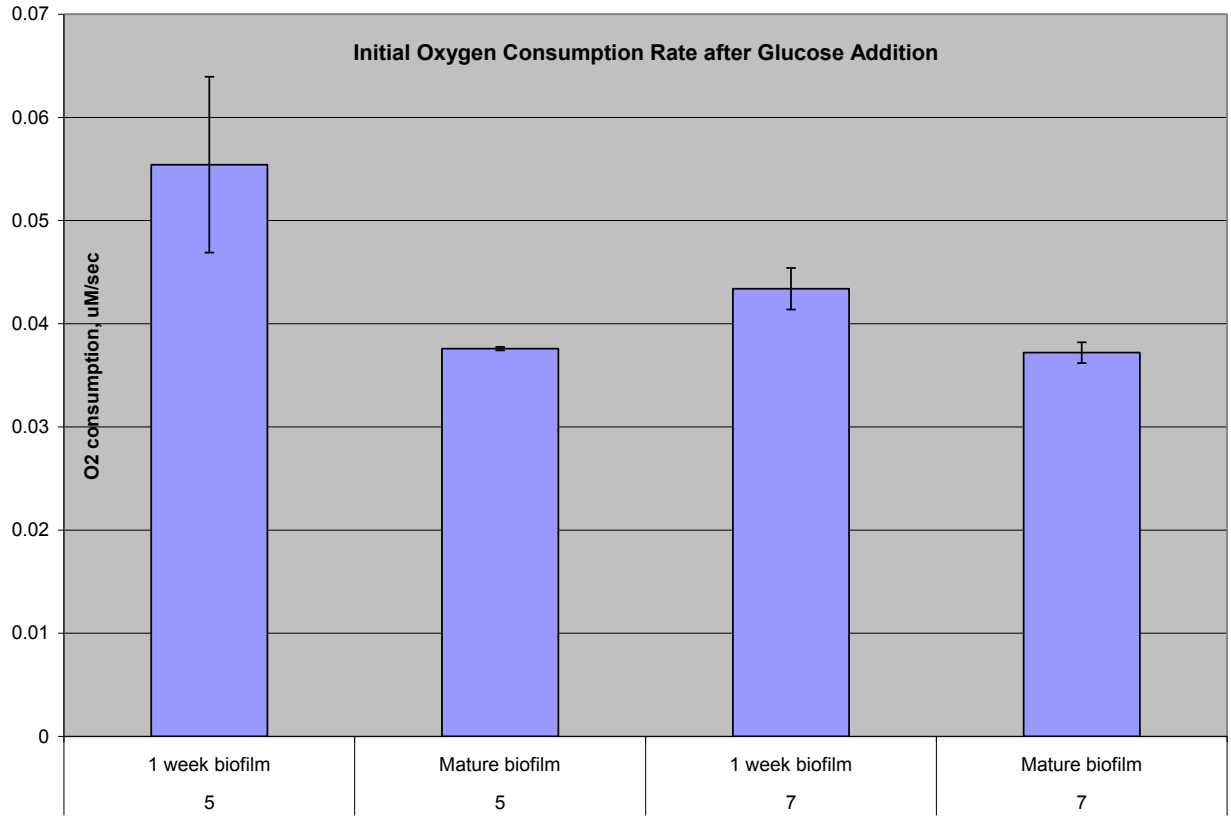


Figure 7: Glucose Metabolism by Biofilms

Conclusions

The biofilms tested from the marine aquaculture trickling filter were not found to be different in species richness, species diversity, or community composition, though a necessary caveat to this analysis is that rarefaction curves generated from the data show that the system has been under-sampled. The similarity between the biofilm communities suggests that biofilm community diversity and structure in marine aquaculture trickling filter systems may be robust and widespread despite differences in temperature and nutrient concentrations.

Colonization experiments also demonstrated that biofilms can metabolize glucose and ammonium after one week of colonization. Utilization of electron donors occurs at levels in the range and in some cases superseding mature biofilms. This suggests that there is a group of colonizing heterotrophs and nitrifiers that may initially colonize a surface and then later be out-competed by more efficient organisms as the biofilm community develops and the available nutrients are depleted.

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