

Evaluation of microbial community structures and coliform persistence in the Alfred Waste Water Treatment Plant Reed Bed Sludge Treatment System

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Summary

The Alfred Wastewater Treatment Facility (AWTF) recently entered an experimental phase of sludge reduction using an uncovered reed bed system. The purpose of this study was to evaluate microbial community response to sludge application, as well as coliform persistence during a six week period of sludge application. Biolog EcoPlate™ analysis of microbial community structure and density measures of bacteria through heterotrophic plate counts were used to determine the impact of sludge application on microbial community structure. Coliform testing determined the pathogen reduction capabilities of the reed bed, which would also provide information on the efficiency of the reed bed since pathogen content is a widely accepted measure of water quality. All data were correlated with physical measures obtained by the AWTF employees. As a result of this study, we determined that species richness and species specialization are reduced immediately following sludge application and do not appear to be affected over the long term. We were also able to determine that, while proficient in pathogen removal, the reed bed sludge treatment system does not remove enough for the left-over sludge to qualify as safe compost according to EPA guidelines. Considering data collected by the AWTF, the current level of sludge application is very efficient at solids reduction. However, less cost to the AWTF and to the environment would result if further research were done to increase coliform removal, which would increase its potential as fertilizer.

Introduction

Flow-through reed bed wastewater treatment systems in the 1990s proved that key pollutants could be removed from water in a low-cost and green manner (2, 10, 11). Their success led to experimentation with the sludge that accumulates in microbially-activated bioreactors. The success of these new reed bed sludge treatment systems lies in their ability to remove heavy metals, Chemical Oxygen Demand, Biochemical Oxygen Demand, NH₄-N, phosphorus and suspended solids (2, 3). Relatively little attention has been paid, however, to the microbes that are significant contributors to reed bed sludge treatment system activity and productivity and the pathogens that are possibly contaminating the effluent.

The presence of pathogens in wastewater is a widely recognized indicator of water quality and the efficiency of the treatment method (4, 9). Although much research has been done on the removal of these pathogens by flow-through beds that treat water, sludge effluent has been largely neglected. 2005 marked the third year of operation of the AWTF reed bed. Reed plants were exposed to increased quantities of activated, aerated sludge as a result of their new status as adults.

The microbial communities that do work in the reed bed itself are considered essential to the proper functioning of the reed bed. Those communities that live in tight symbiosis with the root zones of the reeds have been declared essential for the mineralization of sludge (10). However, in wastewater treatment systems, clogging was a primary factor in decreased performance of reed beds over time. Biofilms and microbial biomass were shown to be a major factor in this clogging (8). They act to decrease aeration and porosity in the sludge and soil. This, in turn, may impact the efficiency of the reeds. This leads to the obvious conclusion that bacteria are useful to a reed bed sludge treatment system to a certain point. After this point, some form of manual population control may be needed to maintain system efficiency.

A useful method of monitoring population density and community structures is community metabolic profiling using Biolog EcoPlate™s. Biolog plates have been used extensively to evaluate the impact of various pollutants on microbial communities, including heavy metals (7, 12, 13). They are also widely used indicators of soil health (1, 12). Therefore, they seem to be an ideal method for analysis of water quality on a community, rather than density, basis. Carbon utilization patterns and kinetics of carbon source usage can be analyzed by several means to determine community species density and evenness.

Monitoring populations by carbon source utilization in conjunction with coliform testing would provide useful measures of microbial activity. This, combined with the physical measures of reed bed sludge treatment system efficiency gathered by the plant workers yields cause/effect data and standard conditions for the reed bed through statistical analyses. Close inspection of microbial diversity allows insight into reed bed sludge treatment system dynamics which would be easily applicable to bettering bed maintenance. This is especially important given the fact that the system is uncovered, which may influence microbial populations, and thereby reed bed sludge treatment system productivity. Therefore, the specific aims of this study were as follows.

- The microbial community structure in the reed bed sludge treatment system was assessed through carbon source utilization patterns. Changes were observed over time and were correlated with sludge application throughout the summer.
- The persistence of indicator pathogens in the reed bed sludge treatment system was determined through coliform analysis.
- Bacterial load was determined through heterotrophic plate counts and direct visualization.
- Data were coordinated and analyzed with physical data collected by the AWTF to provide feedback to the AWTF regarding bed maintenance.

By examining pathogen reduction by the reed bed sludge treatment system in combination with microbial community structure, efficiency trends were characterized and recommendations for improvement made. This has a direct bearing on the efficiency of an energy-saving operation. Reed bed sludge treatment systems are cost-effective because they are low-technology and low energy (4). Also, there is the environmental impact of a reduced load on landfills, which was the initial primary motivation behind the installation of this particular system. Additional environmental impact assessment resulted from examination of pathogen reduction, since the sludge left behind in the reed bed may be used as fertilizer elsewhere. This research explored an important environmental aspect of a relatively new technology, offering potential avenues for efficiency improvement, red alerts if it turns out to be environmentally unfriendly and/or legitimization for a new, effective treatment for sewage pollutants.

Methods

Sampling

The Alfred Wastewater Treatment Plant is located in Alfred New, York, and is the site of an uncovered, 2,500 sq. ft. reed bed with a population of *Phragmites australis*. Sludge is drawn from an aerobic sludge digester and applied directly to the bed. Dates of sludge application were: Jul 1st, 5th, 18th, and 25th, and Aug 2nd and 8th, 2005.

Using the access point from which the secondary sludge flows out over the reed bed, composite influent samples were taken over a period of 10 minutes, with a set volume taken every 30 seconds. A clay auger was used to sample every five feet along the perimeter of the reed bed at a distance of every five feet around the perimeter, 1.5 feet in from the edge. Samples were additionally taken from these same horizontal locations at a depth of one foot and bulked for a composite sub-surface sample. Prior to analysis, solid composite samples were homogenized by manual kneading

for 5 minutes and liquid composite samples were vortexed on high for one minute. Percent solids content of influent sludge as well as pH data were obtained from the Wastewater Treatment Plant.

EcoPlate™ inoculation

Processed samples were immediately used to inoculate EcoPlates™ (Biolog). One half gram of the soil/sediment sample was vortexed in 0.5 mL phosphate buffer on high for 10 minutes, then added to 9 mL phosphate buffer to make the stock for subsequent serial dilutions. Between each transfer and each inoculation, dilutions were vortexed for 30 seconds. EcoPlate™s were inoculated as per Gomez (5) to determine kinetics of carbon utilization. EcoPlate™s were incubated at 28°C and read at 590nm, 7 days after the start of incubation.

Community level metabolic fingerprinting and analysis

Plates were read using an ELISA plate reader at 590nm. Wells with an OD₅₉₀ greater than 0.25 were considered positive for growth. The triplicate data for each dilution was averaged and then divided by 31, the maximum number of carbon sources. This value, designated R, indicated the diversity of the sample. The density of each inoculum was determined by heterotrophic plate counts using Tryptone Agar, as adapted from Noce, Giovanni and Putnins (8). Double reciprocal graphs were produced for each sample date in order to determine R_{max} (a measure of richness) and K (measure of inoculum density at 0.5R_{max}).

Coliform Analysis

The standard membrane filter technique was used to analyze coliform presence in sludge prior to application on the reed bed, sludge from the surface of the reed bed, and subsurface sludge at a one foot depth in the reed bed. This protocol is excerpted from EPA standards (6, 8). Selected colonies from the m-Endo plates were subbed to MacConkey/Sorbitol medium to detect possible pathogenic strains of *E. coli*.

Results and Discussion

Microbial community analysis – In order to understand the effects of community structure on the efficiency of the reed bed, it was necessary to analyze community structures of bacteria within the reed bed in response to sludge application. Community Level Physiological Profiling (CLPP) was performed with the use of EcoPlate™s. The richness of each sample was measured by the number of carbon sources used, and expressed as a percent of maximum. The richness (R) values were plotted against the culturable inoculum density for these samples. The relationship between richness (R) and inoculum density (ID) measured by tryptone agar counts fitted the non-linear model found by Garland and Lehman.

Gomez showed that carbon source utilization of successive 10-fold dilutions of a sample were characteristic of the original sample community structures, as dilution resulted in extinction of utilization of carbon sources (5). The values of R_{max} (maximum richness) and K (the inoculum density at 0.5R_{max}) are therefore used to describe changes in community structure in response to environmental stimuli. Double reciprocal graphs were used to determine R_{max} and K. Values are reported in Table 1.

The R_{max} values for richness peaked in the weeks of Jul 15 and Aug 5 (Table 1). The increase in both K and R_{max} values for the Jul 15 sample date was interesting given that this sample date did not directly follow a sludge application. The increase in response time may have led to the increase in community richness. However, the Aug 5 sample date also resulted in an increase in richness, but without as large an increase in the number of organisms needed to obtain that richness. This suggests that the individual organisms present in the reed bed on Aug 5 have a greater range of

useable carbon sources as opposed to the organisms present on the Jul 15 date. It is clear from these data that the reed bed communities were challenged by the application of sludge on a weekly basis, and that suspension of sludge application for a two week period allowed the community to recover metabolic capacity. It is not clear, however, why the community present on Aug 5 was significantly different from the earlier sample dates.

Table 1. R_{max} and K values for composite reed bed microbial communities.

	K (CFU/gm)	Rmax
8-Jul	0.004	0.31
15-Jul	215.22	1.90
22-Jul	0.23	0.21
29-Jul	0.89	0.66
5-Aug	19.08	2.64

Coliform Analysis – In order to determine the reed bed's efficiency at removal of harmful pathogens, it was necessary to sample at varying depths, as the liquid in this system flowed down prior to draining from the bed. Reed bed samples or sludge influent samples were homogenized and plated for countable colonies onto m-Endo agar. Metallic green colonies were counted as *E. coli*; indicators of coliform content in the reed bed, and adjusted for dry weight of the original sample. Samples from the surface or from the subsurface (1 ft depth) of the reed bed indicated that coliform counts decreased by factors of 10 to 1000 as liquid flowed down through the reed bed (Table 2). However, surface counts consistently exceeded sludge influent counts, indicating that coliforms are not only persisting in the reed bed, but are propagating. Coliform counts in sludge influent remained fairly consistent.

MacConkey Screening – *E. coli* from m-Endo plates were plated on MacConkey/sorbitol medium to determine if potentially pathogenic strains were present. Results indicate that there is a high probability of pathogenic *E. coli* remaining in the sludge, due to presence of sorbitol non-fermenters.

Table 2. Coliform counts in sludge influent and reed bed samples.

Date	Coliform counts		
	Influent (CFU/ml)	Surface (CFU/gm dry wt.)	Subsurface(CFU/gm dry wt.)
1-Jul	7.90×10^4		
8-Jul		1.17×10^6	
15-Jul		2.84×10^6	
22-Jul		5.41×10^5	1.88×10^4
26-Jul	1.42×10^6		
29-Jul		2.79×10^7	1.19×10^6
2-Aug	2.20×10^5		
5-Aug		3.48×10^7	4.77×10^5
9-Aug	2.57×10^5		
12-Aug		6.56×10^6	2.03×10^4

In Conclusion – The AWTP reed bed sludge treatment system has proven itself this summer to be able to handle its current load of sludge. It is unclear whether it will stand up to heavier treatments due to its clear effects on the bacterial community. Additionally, although the reed bed has proven to be efficient at removing pathogens vertically, there are still significant levels of coliforms present in the reed bed due to propagation of the coliform communities at the surface of the reed bed.

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