



THE MICROBIAL QUALITY OF A WETLAND RECLAMATION FACILITY USED TO PRODUCE AN EFFLUENT FOR UNRESTRICTED NON-POTABLE REUSE

R. S. Fujioka, A. J. Bonilla and G. K. Rijal

Water Resources Research Center and Department of Microbiology, University of Hawaii, 2450 Dole Street, Honolulu, HI 96822

ABSTRACT

An auxiliary Wetland Reclamation Facility (WRF) was constructed to receive stabilization pond treated sewage and further treat it with water hyacinth ponds, chemical flocculation, filtration and ultraviolet light disinfection. This was the first facility in Hawaii which was approved to produce the highest quality reclaimed water using alternative treatment schemes. We assessed the effectiveness of the WRF by monitoring water samples after each of the WRF treatment schemes for five genetically different groups of sewage borne microorganisms (fecal coliform, enterococci, *C. perfringens*, FRNA phage, total heterotrophic bacteria). The concentrations of all fecal indicator microorganisms, especially FRNA phage were low in the influent water to the WRF indicating that extended pond treatment may be especially effective in removing human viruses from sewage. The WRF treatment scheme was calculated to be able to reduce >99.99% of fecal coliform and therefore was able to produce an effluent meeting the non-potable, unrestricted reuse standard of a geometric mean of <1 fecal coliform/100 ml. © 1999 Published by Elsevier Science Ltd on behalf of the IAWQ. All rights reserved

KEYWORDS

Fecal coliform; FRNA phage; stabilization pond; water hyacinth pond; water reuse; UV disinfection.

INTRODUCTION

Reuse of wastewater is usually implemented because of insufficient supply of potable water and an economic need for water with lower quality. This is the situation on Lanai, the smallest (364 sq. km) and least populated (~3,000) of the six major islands of the State of Hawaii. Lanai was once called the Pineapple Island because its economy was based on growing pineapple. However, this economy failed and today, the economy of this island is based on up-scale tourism. As a result, two world-class resort hotels and golf courses were built. Initially, the golf courses used potable water for irrigation. This new economy has resulted in an increase in population, an increase in water use and a shortage of potable water supply. As a result, a county ordinance was passed requiring these golf courses to use non-potable sources of water for irrigation. One of these golf courses (The Experience at Koele) is centrally located on the island and the approximately 1,136 m³/d of treated sewage from nearby Lanai City wastewater treatment plant (WWTP) was made available for its use. Since irrigation of the golf course will result in water contact with the public and since the golf course is located above the island's potable ground water sources, the State of Hawaii guidelines require that the sewage be treated to meet the highest quality classification (R-1) for non-potable

uses (State of Hawaii, 1993). To achieve this quality of reclaimed water, it is recommended that sewage be treated using standard sewage treatment methods which includes primary sedimentation, activated sludge treatment followed by chemical flocculation, filtration and finally disinfection with chlorine (USEPA 1992; State of Hawaii, 1993). This recommended series of treatments was devised to produce a final effluent which is essentially free of pathogenic human enteric viruses and consistently contains low to undetectable levels of coliform bacteria. To follow this recommended scheme, the existing Lanai City WWTP which uses stabilization ponds to treat its wastewater would have to be completely renovated at very high cost, especially since the volume of wastewater to be reused is relatively small.

Based on a consulting engineering assessment, The Lanai Company contracted to build and operate a new and relatively inexpensive auxiliary Wetland Reclamation Facility (WRF) and use the reclaimed sewage to irrigate their Koele golf course. The design of the WRF was to accept the effluent from the Lanai City WWTP and further treat this sewage effluent using water hyacinth ponds, followed by chemical flocculation, sand filtration and finally disinfection with ultraviolet light. This is the first facility in Hawaii which uses non-traditional treatment schemes, including the use of water hyacinth ponds, to be approved for production of R-1 quality water. Since there was no preexisting data to indicate the effectiveness of this treatment scheme, the objective of this study was to assess the effectiveness of each step of the WRF treatment scheme in reducing sewage borne pathogens. This was done by analyzing water samples after each of the treatment schemes for the following four genetically different groups of sewage borne bacteria: fecal coliform (gram negative rod), enterococci (gram positive cocci), *C. perfringens* (spore-former), total heterotrophic bacteria (heterogenous group) and a group of fecal bacterial virus (FRNA phage).

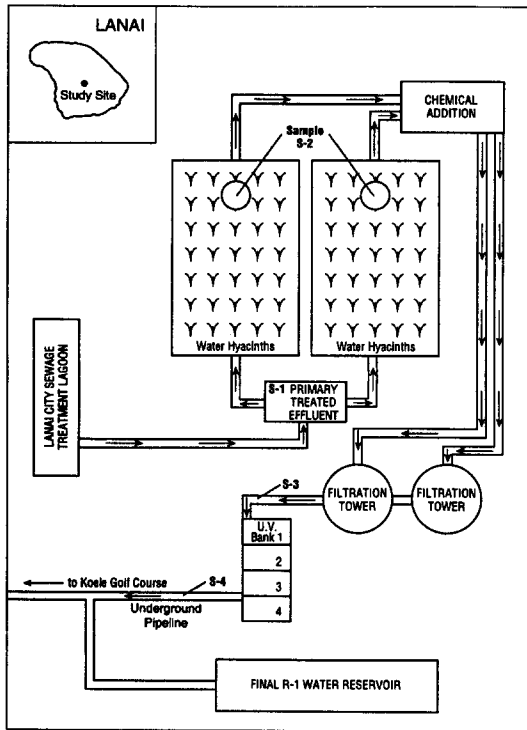


Figure 1. Treatment schemes and sampling sites (S1-S4) at Lanai Wetland Reclamation Facility.

STUDY SITE AND METHODOLOGY

The Lanai City WWTP uses two step (aerobic, facultative anaerobic) stabilization ponds with approximately 50 day retention time to produce 1,136 m³/d of treated sewage with average BOD quality close to secondary

effluent. This final effluent is pumped to the nearby Lanai auxiliary Wetland Reclamation Facility (WRF) where it is the influent that will undergo the following treatment schemes to produce a R-1 quality reclaimed water: 1) Slow flow (9 days retention) through two six foot deep ponds covered with water hyacinths (*Eichhornia crassipes*) to allow the algal cells in the influent to settle out (reduce total suspended solids) and to further reduce the BOD levels. 2) Addition of alum and polymers (Cat-floc) to chemically flocculate organic matter. 3) Upward filtration through sand column to remove the flocculated organic matter. 4) Exposure to four successive banks of low intensity UV lamps (Ultratech Inc.) to disinfect residual microorganisms in the final effluent. The WRF design and sampling points are shown in Figure 1.

Samples from the WRF were obtained using a grab method by personnel of the university but more often by personnel of the WRF and collected into sterile 1 liter plastic bottles which were immediately placed into a cooled ice chest. The samples were promptly taken to the airport and flown to the island of Oahu. Personnel from the university picked up the samples at the airport and transported the samples back to the university where they were analyzed within 8-10 hours of collection. The membrane filtration method as described in *Standard Methods* (APHA, 1995) was used to determine the concentrations of fecal coliform, enterococci and total heterotrophic bacteria while *Clostridium perfringens* was enumerated by the method as described by Bisson and Cabelli (1979). FRNA phage was assayed using the direct agar overlay method or the 100 ml pre-concentration method as described by DeBartolomeis and Cabelli (1991). The concentrations of bacteria were determined as CFU/100 ml while concentrations of FRNA phages were determined as PFU/100 ml. To calculate geometric mean concentrations of microorganisms after each of the treatment schemes, samples which were recorded as 0/100 ml were given a value of 1 and 1 was subtracted from the final cumulative value.

RESULTS AND DISCUSSION

Routine monitoring data by the Lanai City WWTP indicated that raw sewage entering their plant was typical in quality with an average BOD of approximately 200 mg/l. These results were supported by our data which showed that the raw sewage contained normal concentrations (CFU/100 ml) of fecal coliform (6.8×10^7), enterococci (4.1×10^6) and *C. perfringens* (1.4×10^3) as well as FRNA phages (2.2×10^5 PFU/100 ml). The effluent leaving this plant (the influent to the WRF) was determined to have an average BOD of 32 mg/l, indicating that the plant had treated their sewage to a level similar to secondary treatment. However, the total suspended solids concentration of this effluent (approximately 100 mg/l) was high due to algal cell growth in the ponds. Since sewage borne pathogens represent the major health risk in the reuse of sewage (Asano et al., 1992) and since sewage borne pathogens are too numerous and too difficult to assay for, we analyzed 16 water samples (usually monthly) obtained after each of the treatment schemes of the WRF (see Figure 1) over an 18 month period for concentrations of five genetically different groups of sewage indicator microorganisms.

Table 1. Reduction of fecal coliform concentrations after each treatment scheme of the Wetland Reclamation Facility (16 samples over 18 months)

Sample Site	←-----CFU/100 ml-----→				% Reduction
	Range	# 0/100ml/ # samples	Average	Geometric Mean	
Influent	40-460,000	0/16	32,994	2,837	Not applicable
Hyacinth	37-4,400	0/16	760	323	88.60
Filtration	0-18	6/16	4.8	2.2	99.92
UV Disinfection	0-40	14/16	2.5	0.3	99.99

Table 1 summarizes all analysis for fecal coliform and shows that the influent to the WRF had a geometric mean concentration of 2,837 CFU/100 ml and that the water hyacinth treatment reduced that value 88.6% to 323 CFU/100 ml. Following chemical flocculation and filtration, the geometric mean concentration was reduced to 2.2 CFU/100 ml which represented a cumulative 99.9% reduction. After UV treatment, 14/16 samples had undetectable levels of fecal coliform and the geometric mean concentration was reduced to 0.3 CFU/100 ml which represented a cumulative 99.99% reduction. Since Hawaii's R-1 classification for

reclaimed water is based on a geometric mean concentration of <1 fecal coliform/100 ml, the results of this study indicate that the final effluent of WRF meets the R-1 quality.

Table 2 summarizes all analysis for enterococci and shows that the influent to the WRF had a geometric mean of 332 CFU/100 ml and that the water hyacinth treatment reduced that value 83% to 57 CFU/100 ml. Chemical flocculation and filtration removed most of the remaining enterococci as 14/16 samples had undetectable levels of enterococci. The geometric mean concentration of enterococci had been reduced to 0.2 CFU/100 ml which represented a cumulative 99.94% cumulative reduction. After UV treatment, 14/16 samples had undetectable levels of enterococci and the geometric mean concentration of enterococci had been reduced to 0.05 CFU/100 ml which represented a cumulative 99.99% reduction.

Table 2. Reduction of enterococci concentrations after each treatment scheme of the Wetland Reclamation Facility (16 samples over 18 months)

Sample Site	←-----CFU/100 ml-----→				
	Range	# 0/100ml/ # sample	Average	Geometric Mean	% Reduction
Influent	33-1,400	0/16	622	332	Not applicable
Hyacinth	9-272	0/16	94	57	82.83
Filtration	0-5	14/16	0.5	0.2	99.94
UV Disinfection	0-2.5	14/16	0.1	0.05	99.99

Table 3 summarizes all analysis for *C. perfringens* and shows that the influent to the ARF had a geometric mean of 413 CFU/100 ml and that the water hyacinth treatment reduced that value 96% to 15 CFU/100 ml. Chemical flocculation and filtration removed nearly all of the remaining *C. perfringens* as 15/16 samples had undetectable levels of *C. perfringens* and the geometric mean concentration was reduced to 0.04 CFU/100 ml which represented a cumulative 99.99% reduction. After UV treatment, all 16 samples had undetectable levels of *C. perfringens* and this represented a cumulative of >99.99% reduction.

Table 3. Reduction of *Clostridium perfringens* concentrations after each treatment scheme of the Wetland Reclamation Facility (16 samples over 18 months)

Sample Site	←-----CFU/100 ml-----→				
	Range	# 0/100ml/ # sample	Average	Geometric Mean	% Reduction
Influent	40-5,100	0/16	867	413	Not applicable
Hyacinth	0-864	2/16	79	15	96.40
Filtration	0-2	15/16	0.06	0.04	99.99
UV Disinfection	0-0	16/16	0.04	0.00	>99.99

Table 4. Reduction of FRNA phage concentrations after each treatment scheme of the Wetland Reclamation Facility (16 samples over 18 months)

Sample Site	←-----PFU/100 ml-----→				
	Range	# 0/100ml/ # sample	Average	Geometric Mean	% Reduction
Influent	0-9.4	6/16	0.73	0.05	Not applicable
Hyacinth	0-9.2	9/16	1.5	0.3	counts too low to determine %
Filtration	0-0	16/16	0.00	0.00	reduction
UV Disinfection	0-0	16/16	0.00	0.00	reduction

Table 4 summarizes all analysis for FRNA phages and shows that the influent to the WRF had a low geometric mean concentration of 0.05 PFU/100 ml and FRNA phages could not be detected in 6/16 influent water samples. These results show that the Lanai WWTP stabilization ponds were very effective in removing FRNA phages from sewage. The water hyacinth treatment did not measurably reduce the

geometric mean concentration of FRNA phage although the incidence of undetectable levels of this group of phage had increased to 9/16. After flocculation and filtration all 16 samples had undetectable levels of FRNA phage. Due to the low initial concentration of FRNA phages, only a cumulative 99% reduction could be calculated. Similar results (all 16 samples had undetectable levels of FRNA phage) were obtained after UV treatment.

Table 5 summarizes all 14 analyses for total heterotrophic bacteria and shows that the influent to the WRF had a geometric mean concentration of 19,485,233 CFU/100 ml and that the water hyacinth treatment reduced this value 82% to 3,499,636 CFU/100 ml. Following chemical flocculation and filtration, the geometric mean concentration of total heterotrophic bacteria was reduced to 184,971 CFU/100 ml which represented a cumulative 99% reduction. After final UV treatment, the total heterotrophic bacterial count was reduced to a geometric mean of 4,438 CFU/100 ml which represented a cumulative 99.98% reduction. Interpretation of results for total heterotrophic bacteria is difficult because the population of bacteria may be changing after each treatment scheme. However, this was the only group of bacteria which was present in sufficiently high concentrations to reliably demonstrate that the UV treatment can inactivate at least 99% of the bacteria remaining after all of the earlier treatments. It was determined that most of the bacteria surviving UV treatment were resistant spores.

Table 5. Reduction of total heterotrophic bacteria concentrations after each treatment scheme of the Wetland Reclamation Facility (14 samples over 18 months)

Sample Site	←-----CFU/100 ml-----→				
	Range	# 0/100ml/ # sample	Average	Geometric Mean	% Reduction
Influent	3,300,000-850,000,000	0/14	84,345,714	19,485,233	Not applicable
Hyacinth	480,000- 21,000,000	0/14	5,249,286	3,499,636	82.0
Filtration	16,000- 14,000,000	0/14	1,253,264	184,971	99.1
UV Disinfection	1,200- 32,000	0/14	6,886	4,438	99.98

CONCLUSIONS

The influent entering the WRF was determined to contain low geometric mean concentrations (CFU/100 ml) of fecal coliform (2,837), enterococci (332), *C. perfringens* (413) and especially low concentrations of FRNA phages (0.05 PFU/100 ml). Thus, the Lanai City WWTP stabilization ponds system was effective in reducing most of the concentrations of the fecal indicator microorganisms in the sewage. The exceptional removal of FRNA phages by the stabilization pond system was unexpected and may indicate that the long term storage in the open pond system may be effective in removing human enteric viruses. In this regard, WHO guidelines favor the use of stabilization ponds in reclamation of wastewater (Hespanhol and Prost, 1994). Garcia and Becares (1997) previously reported variable removal rates for different microorganisms in stabilization pond systems. However, this could reflect differences in sunlight conditions (Davies-Colley et al., 1997). The fact that the influent to the WRF contained relatively low concentrations of all fecal indicator microorganisms meant that the WRF treatment scheme was required to achieve a cumulative reduction of greater than 99.99% of fecal coliform to achieve R-1 quality. The data showed that the water hyacinth treatment was effective in reducing the concentrations of fecal indicator bacteria by 82-96% but was less effective in reducing the low concentrations of FRNA phage. The chemical flocculation and filtration treatment could be relied on to reduce the concentration of all remaining sewage microorganisms another 90-99% while the final UV treatment is capable of inactivating all remaining sewage microorganisms another 99%.

These conclusions and the successful evaluation of the WRF were possible only because we monitored multiple fecal indicator microorganisms. We therefore, recommend that future studies also include the assessment of multiple groups of fecal indicator microorganisms so one can better compare data and better evaluate the system under consideration. In conclusion, these results show that the WRF treatment scheme to further treat the sewage effluent from Lanai WWTP is effective and can be expected to produce R-1 quality effluent. These results support the use of alternative treatment schemes such as extended stabilization

pond and water hyacinth pond treatment. However, the final treatment schemes, which include chemical flocculation, filtration and disinfection with UV, provide reliability to the WRF process.

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