AN ABSTRACT OF THE THESIS OF

Jan Elaine Morrow for the degree of Master of Science

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Title: COLIFORM BACTERIA ASSOCIATED WITH REDWOOD AND DRINKING WATER EMANATING FROM REDWOOD TANKS

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R. J. Seidler

Coliform contamination of a rural water system led to a research project investigating the problems surrounding storage of drinking water in redwood tanks. Field studies of redwood tanks presently in use showed development of large coliform populations (notably, Klebsiella and Enterobacter species) soon after tanks were put into use. This was often accompanied by development of a fungal slime layer on wood staves below the water line. The presence of a chlorine residual in some field tanks successfully prevented growth of coliforms and resulted in potable water. Absence of a residual allowed growth of coliform organisms and resulted in water unsafe for human consumption.

Studies involving small laboratory sized redwood tanks supported the field observations. Coliforms first develop on the interior staves of a tank, and soon there develops a layer of slime. The coliforms then shed into the water held within a tank unless a suitable chlorine residual is maintained.

Investigation of redwood as the source of coliforms revealed large populations present within the wood structure. Total bacterial counts as high as 10^4/g and coliform counts as high as 10^3/g were obtained using a number of different redwood samples. Both Klebsiella and Enterobacter are present in fresh wood samples, but only Enterobacter persists in older samples. Fresh wood samples from a redwood mill in northern California yielded Klebsiella when incubated in a suitable enrichment medium. This consisted of a modified mFC broth (without rosalic acid) and incubation at 37 C. Coliforms are able to survive in redwood due to growth factors present within the wood structure.

A complete taxonomic study of all coliform isolates was performed. The results were then compared to those published by Edwards and Ewing (8). A greater percentage of Klebsiella isolates were shown to produce indole and H_2S than clinical isolates. Indole production has previously been noted for other environmental isolates (2, 4, 6, 9, 14). Specific compounds present in the water soluble extracts of redwood were also examined as possible growth promoting factors for the environmental coliforms. The carbohydrates and cyclitols (approximately 47% of total water soluble extracts) were shown to be widely utilized as growth factors by Klebsiella and Enterobacter isolates. The phenolic compounds (35% of total water soluble extracts) were shown to be inhibitory to all isolates

at the concentrations used. The presence of these compounds in the redwood and their subsequent leaching into the tank interior provide a suitable environment for the growth of coliforms.

The culmination of this study involved the construction and maintenance of a 1000 gallon redwood water system following guidelines observed in the earlier studies. This tank was provided with a calibrated source of chlorine amendments that could be regulated as needed, and provisions for any number of desired retention times. Need for a suitable period of leaching of growth promoting factors and also for periodic sterilization of the tank interior to prevent slime and coliform development were seen. This system has been in operation for over 7 months with no incidents of coliform contamination of the water.

Coliform Bacteria Associated with Redwood and Drinking Water Emanating from Redwood Tanks

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Errata

The following corrections/changes should be noted.

Abstract - First paragraph, last sentence. The water is unsafe in that it exceeds Federal and Oregon limits for coliforms in drinking water.

Results - page 11, paragraph 2, line 2. Should read, "constructed of old growth heartwood which comes from trees 300 to 1500 years in age."

Abstract - page 23, line 8. Liner was composed of polyvinyl chloride (PVC). PVC should replace the word polyethylene on pages 27, 39, 41, and 46.

LIST OF TABLES

LIST OF FIGURES

COLIFORM BACTERIA ASSOCIATED WITH REDWOOD AND DRINKING WATER EMANATING FROM REDWOOD TANKS

INTRODUCTION

In the summer of 1974, a rural drinking water system located near Albany, Oregon developed water quality problems. Coliform counts were in excess of those permitted by federal standards and the water was declared unsafe for human consumption. The water storage tank was constructed of redwood which had been manufactured by National Tank and Pipe Company of Portland, Oregon. The vice president and general manager of this firm, Jack Behrens, was contacted concerning the water problem. He, in turn, contacted Dr. R.J. Seidler, associate professor of microbiology at Oregon State University, Corvallis. A preliminary investigation of the tank system was undertaken.

The incoming water (from three wells) was examined and found to be coliform-free. There was no evidence of any outside contamination from insects, rodents, or birds, and thus the tank itself was suspected as the focal point of the coliform problem. Coliforms isolated from the tank water were species of the genera Klebsiella and Enterobacter. As a result of this preliminary work, a research program concerning the association of coliforms with redwood tanks was initiated between National Tank and Pipe and our research group at Oregon State.

This problem of coliforms associated with water emanating from redwood water tanks was by no means a new one. Mr. Behrens communicated that a number of the tanks sold by National had similar problems, which led to the need for a more thorough investigation.

Only one report of a similar problem existed in the literature, this being a report concerning redwood water storage tanks in northern California (12). That study was descriptive in nature; there was no mention of coliform counts found nor the genera involved. Hence, the basic problem of coliform contamination of redwood tanks was documented but the present problems in Oregon indicated additional studies were necessary.

The primary purpose of the present study was to investigate the coliform problem as it pertained to the manufacturer's needs, namely, the occurrence of coliforms in redwood water reservoirs, causes of this problem, and ways to control it.

Klebsiella pneumoniae has always been something of an enigma to bacteriologists. First isolated in 1882 as Friedlander's bacillus, it was classically restricted to respiratory infections in humans (5). In the early 20th century, a genus of bacteria, Aerobacter, was used in sanitary water analyses as being indicative of organisms of an unpolluted environment. However, the cultural descriptions of both Klebsiella (as Friedlander's bacillus was named) and Aerobacter were

identical. The only differentiation of the two genera was based on habitat of origin (5).

In 1929, a number of supposed Aerobacter isolates from the soil were agglutinated with antiserum prepared against Friedlander's bacillus (Klebsiella) (7). It was not until 1960 that Edwards of the Center for Disease Control developed reliable biochemical tests to differentiate the two genera. At this time, he renamed Aerobacter as a new genus, Enterobacter (5).

While Klebsiella pneumoniae is today primarily recognized as the cause of numerous types of nosocomial infections, it is also associated with many habitats in the environment. The organism has been found in association with vegetables and vegetable seeds (4), effluents from pulp, paper, and textile mills (17), and present as nitrogen fixing strains in association with living white fir trees (2).

Klebsiella is also the cause of mastitis in dairy herds, and reported to originate from sawdust used in the dairy barns. Removal of the sawdust is accompanied by a decrease in infection (15).

All Klebsiella, regardless of origin, conform to the operational definition of coliforms, and hence must be excluded from drinking water systems. They are carried in the human and animal intestinal tract, and therefore are potential indicators of possible fecal contamination.

The Oregon Department of Human Resources, Health Division, which is responsible for testing and granting permits for water systems, has discouraged the further installation of redwood water storage tanks until the coliform problem and its possible significance is resolved.

The early stages of this research investigation involved two areas of interest: examination of the nature of possible coliform problems in redwood tanks in current use, and the development of a wooden water storage system which would yield potable water. Chapter I presents the results of a study of field tanks located in the state of Oregon. A questionnaire to water purveyors produced a history of each water system and any related problems. Receipt of the questionnaire was followed up by actual sampling visits to many of the tanks to examine the problem as it existed.

The investigation of laboratory tanks is detailed in Chapter II. Three 65 liter tanks were constructed by National Tank and Pipe and sent to the Oregon State University laboratory. These three tanks were examined extensively to study the patterns of development of the coliforms, and also to explore various possible solutions to maintain high water quality.

The next area of study was the origin of coliforms found in the tank water. This is discussed in Chapter III. Redwood was thoroughly examined as the point source of the coliforms. This was done by

expressing liquid from redwood blocks, examination of sawdust and chips, and by direct impression of wood surfaces onto various nutrient media. All of these experiments were attempted to show that the coliforms are located in the redwood itself.

As coliform organisms were isolated in the earlier parts of the work, an obvious need developed to do a complete identification of these isolates. These procedures and results are described in Chapter IV. Besides an examination of all pertinent biochemical reactions, various compounds present in redwood itself were examined for possible sources of nutrients for the coliforms.

The culmination of this investigation is described in Chapter V. This chapter deals with the construction and maintenance of a 1000 gallon redwood tank to see if such an experimental system could be successfully managed using information acquired in the previous chapters. Investigations of problem-ridden redwood systems in the field had incriminated a number of specific trouble areas such as long retention times, insufficient chlorine residual at all points on line, insufficient circulation of the water, and incomplete preliminary sterilization of the tank interior. Parameters were set up in response to these problems, and a 1000 gallon tank was carefully maintained and observed from original construction to final on-line status.

FIELD SURVEY OF REDWOOD WATER SYSTEMS

Abstract

Twelve operational redwood water systems were examined at a number of sites located in western Oregon. Water samples were examined for three criteria: 1) total number of microorganisms present, 2) total number of coliforms and species of coliforms present, and 3) total chlorine concentration. The genera Klebsiella and Enterobacter were the organisms commonly found in the coliform-positive water samples. A lack of chlorine in the water was a common occurrence. Two water systems with detectable chlorine yielded coliform-free water. Data presented show that a number of factors result in unsafe drinking water when judged by either state or federal standards. These factors include: absence of total chlorine residual, long retention time of water, and poor circulation due to unsatisfactory plumbing.

Materials and Methods

Sampling of Field Tanks

Operational redwood water systems were examined at a number of sites located in western Oregon. These tanks were located in Corvallis, Eugene, Reedsport, and Klamath Falls. Whenever possible, the water sample from a field tank was taken directly from the interior of the tank. This was accomplished by climbing a ladder on the tank exterior and entering through a hatch on the tank roof. If the water level was high, a sample was taken by submerging a sterile 1000 ml Erlenmeyer flask under the water surface. In other cases, the flask was lowered to the level of the water using a rope. If the tank was not accessible, a sample was taken from the most available spot on the service line. The flasks were closed with a piece of sterile foil, the samples stored on ice and processed as soon as possible. This was anywhere from two hours (Corvallis) to 36 hours (Klamath Falls).

If a sample of slime was taken, it was obtained by scraping a small sample from the surface of the tank interior with a sterile glass slide. This sample was then stored in a sterile beaker containing 10 ml of sterile distilled water added to prevent desiccation of the sample. The beaker was covered with sterile foil and placed into an ice chest.

Most information concerning plumbing, chlorine regimens, and general maintenance was obtained from questionnaires sent by National Tank and Pipe Company, Portland, Oregon to tank operating personnel (Jack Behrens, personal communication). Other information, such as the current status of the tank and any recent problems, was received from these personnel at the time the tank was sampled.

The total chlorine concentration (ppm) was checked for each tank sampled. This was tested by the orthotoluidine method (16). Readings as low as 0.1 ppm total chlorine can be detected by this method.

Processing of Water Samples

Water samples from each tank were examined for three criteria: 1) total number of microorganisms present, 2) total number of coliforms and species of coliforms present, and 3) total chlorine concentration.

To obtain total numbers of microorganisms present, Bacto Nutrient Agar was used as a non-selective isolation medium. Fifty ml of each sample were filtered using a Millipore filtration apparatus (pore size 0.45 μm), and filters placed on the agar surface. Dilutions of 10^{-1}, 10^{-2}, and 10^{-3} were also plated, using the spread plate technique. All plates of nutrient agar for total counts were incubated at 30 C. Counts were made after 24 and 48 hr incubation.

For total coliform counts, Bacto m-Endo LES agar was used as a selective medium for the isolation and enumeration of coliform organisms. Ten and 100 ml samples were filtered and, in addition, a direct streak plate (one loopful of sample) was also prepared. These plates were incubated at 35 C, and counts were made after 24 and 48 hr.

In addition to the membrane filtration for total coliform counts, the MPN technique was also used in one sample. Double and single strength Bacto phenol red lactose broth was used in this procedure. Ten ml of sample were put into each of five tubes of double strength broth, 1 ml into each of five tubes of single strength, and 0.1 ml into each of five tubes of single strength broth. These tubes were incubated at 35 C and examined for the production of gas after 24 and 48 hr.

When samples of slime were obtained, these were examined for the presence of coliforms within the structure of the slime itself. One loopful of the liquid was streaked onto nutrient agar for total microorganisms present, m-Endo LES agar for total coliforms, and Bacto malt agar for yeasts and molds. The nutrient agar and malt agar plates were incubated at 30 C and the m-Endo LES agar at 35 C. Results were read after 24 and 48 hr.

Identification of Isolated Organisms

Coliform isolates were presumptively identified by the presence of green sheen colonies on m-Endo LES agar. When the MPN technique was used, loopfuls from positive tubes were streaked onto m-Endo LES to obtain isolated colonies. All presumptive coliforms were then inoculated into Bacto TSI agar slants to check for fermentation, H_2S formation, and gas production from lactose, sucrose, and glucose. These were incubated at 35 C for 24 hr.

Isolates were then inoculated into media to check for the presence of lysine and ornithine decarboxylase, and arginine dihydrolase activities. This medium consisted of Bacto Moeller's Broth Base and the appropriate amino acid. These were incubated at 35 C and read each day for 4 days. In a few instances, Enterotubes (Roche Diagnostics) were used to obtain further information. Media preparation and identification procedures were those of Edwards and Ewing (8), and Lennette, Spaulding, and Truant (13).

Presumptive coliform isolates were stored on nutrient agar slants at 5 C for further taxonomic studies. Yeast and mold isolates were maintained on malt agar slants at room temperature until appropriate identification could be arranged. Details of further taxonomic studies are presented in Chapter IV.

Results

Table 1-A shows the results of a field survey on redwood water storage tanks currently in use within the state of Oregon. Most of these tanks serve small rural areas such as mobile home parks, Oregon state parks, or small housing developments not supplied by municipal service. Among those tanks serving residential areas, these was considerable variation in the number of households served per system. Large variations were also seen in how each tank system was monitored, there being no state or federal requirements pertaining to system serving less than ten users (1).

The redwood tanks built by National Tank and Pipe are constructed of second growth heartwood which comes from trees 30 to 60 years in age. The mills use heartwood material which is generally taken from the lower portions of the trees. The staves are air-seasoned or kiln-dried before final assembly in the field. Several of the tanks examined (KRA and PW) were made by Sauers Forest Products, Pacific Wood Tank Division, Healdsburg, California. Their tanks are almost identical to those of National Tank and Pipe, and are assumed to be of similar construction.

The information obtained on each tank system came from questionnaires sent out by National Tank and Pipe, and also from direct communication with those persons responsible for the

maintenance of each water system. A sample questionnaire is shown in Figure 1-A.

Three examples from these redwood systems will illustrate the disparity in the way they have been handled.

Tank KRA is located in a residential housing area; Klamath River Acres, Keno, Oregon. This tank has a capacity of 150,000 gallons; the water source is a well. It is of redwood construction with a concrete base. This tank was first filled with water in September of 1974, and chlorine was added to bring the solution to 50 ppm. This remained in the tank for 24 hr, as recommended by the manufacturer. The tank was drained and then refilled to 70% capacity. This same water was standing in the tank when it was examined in January of 1975. The housing development was scheduled to open later that year, but at the time of examination no homes were occupied.

A water sample was taken from the top of the tank through a hatch on the roof; a strong coliform odor was noted at that time. No samples of slime were obtained. The MPN test yielded > 1100 coliforms/100 ml (5-5-5 positive tubes), membrane filtration using m-Endo LES gave 200 coliforms/100 ml, and nutrient agar gave total counts of 200/100 ml. Three organisms picked from the m-Endo LES plate were identified as Klebsiella pneumoniae. This was later confirmed by further taxonomic testing.

WATER SYSTEM STUDY - QUESTIONNAIRE

Please complete and return to:

National Tank & Pipe Co.
P.O. Box 17158
Portland, Oregon 97217

1. Is water source a well _____ or other (specify) _____.

2. What is the construction of the tank in use?

Concrete _____ Steel _____

Redwood _____
Wood bottom _____
Concrete bottom ____

3. When was the tank first filled with water?_____.

4. Was the tank sanitized before going into service _____, and what procedure was followed? _____

5. When did the system go into service? _____

6. Was the system chlorinated from the start of service? _____ What type of chlorination equipment was installed _____ _____, and what treatment procedure and schedule was followed? _____ _____

7. If chlorination was not begun at the time of start-up, is the system now chlorinated? _____ When was chlorination begun? _____ What type of chlorination equipment was installed? _____ _____

Figure 1-A. Sample questionnaire used in obtaining information on field tanks.

What treatment procedure and schedule was followed? _____

_____

Why was chlorination begun? _____

_____

8. Is this system in service now? _____

9. Where is the exact location of the tank? _____

_____

Should anyone be contacted before visiting the tank to take a water sample? _____

Contact's Name _____

Contact's Phone No. _____

Contact's Address _____

_____

_____

Figure 1-A. (Continued)

Table 1-A. Field survey of redwood water tanks.

Identification	Location	Capacity	Water use	Age tank (months)	Automated chlorination	Cl_2 residual	Common inlet/ outlet	User households	Number coliforms present	Identification
WP[d]	Corvallis	25,000	Mobile home water supply	17	Yes	0.3 ppm	No	20	None/100 ml m-Endo LES coliforms in slime	Klebsiella
PD[d]	Corvallis	25,000	Residential	10	Yes	0.3 ppm	No	30	None/100 ml m-Endo LES	
EB	Eugene	6,000	Residential	48-72	a	None	Yes	4	2/100 ml m-Endo LES	Enterobacter
EC	Eugene	50,000	Residential	24-48	a	None	Yes	50	2/100 ml m-Endo LES	Klebsiella, Enterobacter
EA	Eugene	50,000	Residential	10	a	None	Yes	50	15/100 ml m-Endo LES	Klebsiella, Enterobacter
DA	Reedsport	10,000	Recreation	48	Yes	None	Yes	Varies widely	None[b]	
SC	Reedsport	10,000	Fire storage only	Old	No	None	Yes	None	10/100 ml m-Endo LES	Klebsiella, Enterobacter
SLV	Klamath Falls	50,000	Residential	20	No[c]	None	Yes	68	150/100 ml m-Endo LES MPN >1100/100 ml	Klebsiella
MH	Klamath Falls	50,000	Residential	19	No[c]	None	Yes	50	200/100 ml m-Endo LES MPN >1100/100 ml	Klebsiella, Enterobacter
KRA	Klamath Falls	150,000	Residential (not yet in use)	3	No	None	Yes	None	200/100 ml m-Endo LES MPN >1100/100 ml	Klebsiella
PW	Klamath Falls	50,000	Residential	3	Yes	None	Yes	4	80/100 ml m-Endo LES MPN 150/100 ml	Klebsiella
BW	Klamath Falls	50,000	Residential	5	Yes	None	No	5	150/100 ml m-Endo LES MPN >1100/100 ml	Klebsiella

[a]Water enters tank via city pipes at 0.2-0.4 ppm chlorine residual. No further chlorine treatment at tank site.

[b]Tank not directly accessible; sample removed from faucet about 1 mile from tank.

[c]Tank occasionally hand chlorinated with tablets only. Reportedly "no recent problems." No chlorine residual at time of sampling.

[d]Only tanks with constant automated chlorine treatment and modest use (about 7 day turnover) give a potable water analysis.

The second tank, part of the Bley Was Heights area in Bly, Oregon, was also examined in January of 1975. The system was put into service in October of 1974 and served five homes at the time of sampling; more were expected later. This was a redwood tank with concrete bottom, water source a well, and a capacity of 100,000 gallons. There were separate inlet and outlet water sources, the inlet being located at the top of the tank and the outlet located at the bottom.

The original treatment consisted of a solution of 50 ppm chlorine for 24 hr. A gas chlorinator had been used since service began, the water being chlorinated at the well before going to the tank for holding. There had been complaints of bad tasting water by users. Retention time was estimated at 33 days.

At the time of examination, the tank was filled at 75% of capacity. A water sample was obtained from a sampler located on the side of the tank. Slime samples were taken from the tank interior near the top. Some staves were heavily covered while others were barren. There was no total chlorine detected by the OTO method.

The results of analysis of this water showed >1100 coliforms/100 ml (5-5-5 positive tubes) using the MPN method, 150 coliforms/100 ml on m-Endo LES, and total counts of 1000/100 ml on nutrient agar. One isolate was confirmed as Klebsiella pneumoniae.

The third tank is located near Corvallis, Oregon in the Whispering Pines Mobile Lodge. It is a redwood tank with concrete bottom, water source a well, capacity of 25,000 gallons. There are approximately 20 user households. The tank was first filled with water in November of 1973. It was initially treated with 50 lb of Sal Soda (soda ash) for 24 hr in a full tank. This was followed by 12 gallons of a 10% chlorine solution added to a full tank. This remained in the tank for 2 weeks. The tank was again filled with 6 gallons of a 5.25% sodium hypochlorite solution added to a full tank and this was held in the tank for 24 hr. The system went into operation in late January of 1974.

The water drawn from the well enters a pumphouse where chlorination occurs. The chlorine is added via a Wallace-Tiernan solution metering pump (Model 94-100). The chlorine solution used is a 15:1 water to laundry bleach mixture (sodium hypochlorite at 5.25%). The water then travels a few feet to the redwood tank for storage. The water enters the tank at an inlet 6 inches from the bottom and leaves by a separate outlet on the opposite side of the tank, also located 6 inches from the bottom.

This system has been monitored extensively by the operator, including daily examination of amounts of chlorine entering the water at the pumphouse and the amounts reaching users on line. The total chlorine was checked using a test kit employing the OTO method.

Approximately 2 weeks after the system was put into operation, users complained of taste, odor, and red coloration of the water. A total chlorine concentration of 0.2-0.3 ppm was maintained, and a water quality check was negative for coliforms. On a suggestion from National Tank and Pipe, 50 lb of Sal Soda was added to a full tank to further remove phenolic and tannin compounds leaching from the wood. The taste, odor, and coloration problems continued for some weeks, but by July of 1974 had almost disappeared. At all times a chlorine residual of 0.2 ppm had been sustained, and all water quality examinations had been negative for coliforms. Accumulation of slime appeared sporadically on some staves, at about 6 months and 12 months after initial operation. This was removed by draining the tank and scraping the staves clean using a concentrated chlorine solution. The turnover time of this tank is usually 4-6 days, slightly higher in the summer and slightly lower in the winter.

When the tank was examined in December of 1974, there were again deposits of slime on some staves. A sample was taken, and microscopic examination showed a fungal mycelial matrix with bacteria embedded therein. A qualitative examination of this slime showed coliforms present. These were confirmed as isolates of Klebsiella pneumoniae. The water itself yielded 0 coliforms/100 ml and total bacterial counts of 100/100 ml on nutrient agar. The water had a chlorine residual of 0.2 ppm.

Discussion

The basic problem associated with these representative redwood water tank systems is that most of them do not meet federal or state standards for drinking water quality. The United States Public Health Service Standards of Drinking Water Quality states the following:

> The maximum number of allowable coliform organisms is prescribed in terms of standard portion volume (10 ml or 100 ml) and the number of portions examined. The absence of gas in all tubes, when five 10 ml portions are examined by the fermentation tube method (less than 2.2 coliforms per 100 ml), is generally interpreted to indicate that the single sample meets the standards. A positive Confirmed Test for coliform organisms in three or more tubes (10 ml portions), or the presence of four or more coliform organisms in 100 ml samples established by membrane filter tests, indicates the need for immediate remedial action and additional examinations (16).

The state drinking water standards for Oregon are even more rigorous, as stated in the Oregon State Health Division Administrative Rules for Domestic Water Supply Systems:

> (A) When the membrane filter technique is used (100 ml sample size), the total coliform densities shall not exceed one per 100 ml as the arithmetic mean of all samples examined per month; and either: (i) four per 100 ml in more than one standard sample when less than 20 are examined per month; or (ii) four per 100 ml in more than five percent of the standard samples when 20 or more are examined per month. (B) When the multiple tube fermentation method (10 ml standard portions) is used, total coliform organisms shall not be present in more than 10 percent of the portions in any month; and not be present in either: (i) three or more portions in more than one sample when less than 20 samples are examined per month, or (ii) three or more portions in more than five percent of the samples if more samples are examined per month (1).

Most of the tanks examined in this survey do not conform to either federal or state of Oregon drinking water specifications by either the MPN or membrane filtration techniques.

In the non-conforming tanks, the poor water quality is associated with the absence of a chlorine residual. Essentially all except two tanks had no total chlorine detectable when sampled. However, if a suitable residual (0.2-0.3 ppm) is present, no coliforms are seen (as in the WP and PD tanks). Furthermore, tanks with no provision for steady chlorination (i.e., those tanks where chlorine tablets are added irregularly by hand) show no lasting chlorine residual. Redwood appears to have a high chlorine demand of its own, further reducing chlorine residual.

A higher chlorine concentration needs to be maintained within the incoming tank water than is realized by many water purveyors. In addition, adjustments must be made for varying retention times. This concentration appears to be at least 1.0-1.5 ppm, as observed in the WP tank, in order to maintain a desirable residual of 0.2 ppm in the outgoing water. Also, if a tank has a common inlet and outlet there is little mixing of the water; this is also true if the inlet and outlet are separate but both at the tank bottom. There needs to be a distance (as from top to bottom of tank) between these two openings to promote adequate mixing of the incoming chlorinated water with the water already present in the tank.

The appearance of a fungal slime is also a problem in these tanks, as it apparently serves as a protective niche for coliforms (as in WP). It also can cause problems with plumbing systems as it tends to slough off in large pieces. The fungal matrix, covered with a relatively thick layer of slime, evidently protects the coliforms from encountering any chlorine that may be present in the water and may in itself contribute to the chlorine demand.

The problems of taste, odor, and coloration of the water are due to the phenolic compounds (and chloramines) that leach from the redwood, and are probably related to organic chlorine complexes formed. This situation is most severe when a system is put into use for the first time, and decreases as the tank ages and compounds leach out entirely. An adequate pretreatment is needed to get rid of these compounds before the water is actually used for human consumption. The soda ash treatment currently recommended by the manufacturer seems to be somewhat effective, but longer and repeated treatments may be needed to remove additional traces of the compounds involved. This pretreatment should also serve to retard the development of the slime.

In summary, a water system using a redwood tank can be satisfactorily maintained only if certain precautions are taken. The plumbing for the system should provide for separate inlet and outlet openings, so that adequate mixing of the water and chlorine can occur.

Pretreatment of the assembled tank should include an extended soda ash soak followed by sterilization of the interior by concentrated chlorine solutions held for 2-3 days. This should serve to counteract early emergence of a fungal slime layer and coliform bacteria. Chlorination of the water should occur in the tank itself, with suitable mixing and very regular and thorough monitoring. This monitoring should make provisions for varying turnover times such that a residual of 0.2 ppm chlorine is maintained within the tank and at all points of the system.

If conscientious efforts are made at constructing and running a redwood tank system, it should be a very suitable and durable system that will provide safe, clean drinking water for many years. However, these actual parameters of chlorine amendments, limits of retention times, and necessary plumbing adaptations are not well understood for redwood tanks on line in the field. Further studies within the research laboratory and a large 1000 gallon experimental redwood tank system should serve to clarify the interaction of these various parameters.

EXAMINATION OF EXPERIMENTAL REDWOOD WATER SYSTEMS

Abstract

Three experimental redwood water tanks were examined for the development of coliform problems as seen previously in the field systems. Coliforms were seen to first develop on the staves of the tank, generally in conjunction with a fungal slime layer. The organisms then move to the water contained within the tank. The genus Klebsiella was shown to have no unique resistance to chlorine and specific chlorine amendments were seen to control coliform development. A polyethylene liner was shown to greatly reduce loss of chlorine residual, thus demonstrating a high chlorine demand exhibited by redwood itself. Appropriate chlorination and circulation of the water within an experimental tank was shown to adequately control the coliform problem.

Materials and Methods

Experimental Laboratory Redwood Tanks

Figure 2-A shows one of three experimental redwood tanks built by National Tank and Pipe especially for the present laboratory investigations. The small tanks were constructed on the same basic design as the larger field tanks. However, there were no openings made to serve as either inlet or outlet. The capacity of each tank was approximately 65 liters. A flat plywood lid was fitted over the top of the tank.

Each tank was 23 inches high, 24 inches in diameter, and 80 inches in circumference. Each was composed of 22 redwood staves that were $2\frac{1}{2}$ by $3\frac{1}{2}$ inches. Iron hoops encircled the tank to seal the staves in place. Tank A was constructed of heartwood that had been kiln dried at 180 F. Tanks B and C were also of heartwood, but were air dried. These tanks were built at the Portland plant and transported to the OSU laboratory in the summer of 1974.

Monitoring of Laboratory Tanks

To check the water quality within each tank, periodic chemical and microbiological examinations were made. In order to enumerate the total bacterial flora of the tank water, samples were diluted and spread plates of suitable dilutions were made on non-selective Bacto

Figure 2-A. Experimental redwood tank, 65 liters.

nutrient agar medium. These plates were incubated at 30 C for 24 hr. For total coliform counts, water samples were filtered using a Millipore filtration apparatus (pore size 0.45 μm). The filters were placed onto m-Endo LES agar and incubated at 35 C for 24 hr. To examine the development of slime on tank staves, sterile glass slides were used to scrape the tank interior. The slime was diluted in sterile distilled water and the resulting liquid was either filtered or streaked directly onto both nutrient agar and m-Endo LES agar.

Any mold growth occurring on nutrient agar plates was isolated on either Bacto potato dextrose agar or Bacto malt agar. These plates were incubated at 30 C for 24-48 hr. Mold and yeast isolates were stored on slants of malt agar and held at room temperature.

Chlorination Scheme for Tank B

After preliminary examinations, the interior of Tank B was scrubbed with a solution containing 1000 ppm total chlorine. Chlorination was then begun; this was accomplished using Hth granular dry chlorine (65% hypochlorite) as the source of chlorine. The amount needed to produce the desired final concentration was weighed out, then added to the tank in concentrated solution as the water was agitated. All water added to the tank came from Corvallis city tap water via a rubber hose (added to top of tank). When the tank was to be drained, a piece of hosing was again used as a siphon drain. After

the tank was full, the lid was replaced and water left undisturbed until the next examination. The water quality was monitored using the procedures described above. The chlorine residual of the water was checked using a Guardex pool testing kit employing orthotoluidine as the indicator agent. The pH of the water was checked with a pH meter, and the optical density examined using a Spectronic 20 at 550 nanometers with tap water as a blank.

Polyethylene Liner for Tank B

A special liner made of polyethylene was sent from National Tank and Pipe for use in control of coliforms and chlorine maintenance in a small tank. This liner resembled a large plastic bag, fitted snugly inside the tank and was fastened around the top.

Modifications for Running Water in Tank B

Figure 2-B illustrates the appearance of Tank B for these experiments involving a running water modification. An outlet hole was drilled through the tank $1\frac{1}{2}$ inches from the top, while an inlet opening was located one inch from the bottom. A piece of rubber tubing at the bottom valve was connected to a Corvallis water tap. Water entering from the bottom forced overflow water through the valve at the tank top and kept circulation at a maximum.

Figure 2-B. Experimental redwood tank with modifications for circulation of chlorinated water.

Viability of K. pneumoniae in Chlorinated Municipal Water vs. Distilled Water

Cultures of Klebsiella pneumoniae from both environmental and clinical sources were used. Cells were grown in Bacto nutrient broth for 20 ± 1 hr, then diluted using sterile distilled water as the diluent. Assuming an initial cell density of 10^9 cells/ml, dilutions were made to final concentrations of 10^2, 10^1, and 10^0 cells/ml. One-half of the samples were diluted into sterile distilled water, while the other half was placed in Corvallis tap water which had a total chlorine concentration of 0.2-0.4 ppm. Contact times of 0, 30, and 60 min were performed. Ten-ml samples were then filtered using membrane filtration, and filters placed onto m-Endo LES agar. These plates were incubated at 35 C for 24 hr.

Identification of Colifirm Isolates from Small Laboratory Tanks

Coliform isolates were presumptively identified by the presence of green sheen colonies on m-Endo LES agar. These isolates were then inoculated into Bacto TSI agar slants to check for fermentation, H_2S formation, and gas production from lactose, sucrose, and glucose. These were incubated at 35 C for 24 hr. Isolates were then inoculated into media to check for the presence of lysine and ornithine decarboxylase, and arginine dihydrolase activities. This medium consisted of

of Bacto Moeller's Broth Base and the appropriate amino acid. These were incubated at 35 C and read each day for 4 days. Any Klebsiella or Enterobacter isolates were saved on nutrient agar slants at 5 C for further taxonomic studies. Media preparation and identification procedures were those of Edwards and Ewing (8), and Lennette, Spaulding, and Truant (13).

Results

Preliminary studies were run on each of the three tanks to qualitatively and quantitatively examine the development of coliform organisms. The results of these studies are shown in Table 2-A. The work on Tank A (kiln dried) was begun earlier as it was the first to arrive at the OSU laboratory. Tanks B and C (air dried) were received approximately two weeks after Tank A.

Tank A was filled upon arrival with distilled water. The tank was then examined after 24 hr; no coliforms were found either by stave scrapings or by water quality examinations. It was then drained and refilled with Corvallis tap water containing 0.4 ppm total chlorine (OTO method). On day 4 of this study, one liter of water was examined that averaged 1.1 coliforms/100 ml (one-liter sample

Table 2-A. Preliminary examinations of laboratory Tanks A, B, and C for development of coliforms.[a]

	Day	Test	Observation	Identification
Tank A	1	1 liter filtered	No coliforms	
	3	1 liter filtered	No coliforms	
	10	300, 100, 5, and 1 ml filtered	No coliforms	
	14	1 liter filtered	No coliforms	
	16	500 ml filtered	No coliforms	
	21	1 liter filtered	No coliforms	
	30	100 ml filtered	1 coliform	K. pneumoniae
	42	100 ml filtered	No coliforms	
		10^{-3}, 10^{-2} dilutions	3000/ml total counts	
	43	100 ml filtered	No coliforms	
	48	100 ml filtered	No coliforms	
		10^{-3}, 10^{-2} dilutions	4500/ml total counts	
	50	10^{-3}, 10^{-2} dilutions	9500/ml total counts	K. pneumoniae
Tank B	1	1 liter filtered	5 coliforms	
	3	Staves scraped	Coliforms present	
	5	1 liter filtered	No coliforms	
	7	Staves scraped	No coliforms	
	8	500 ml filtered	1000 coliforms	E. agglomerans
	22	100 ml filtered	80 coliforms	K. pneumoniae
	34	50 ml filtered	24 coliforms	
		10^{-3}, 10^{-2} dilutions	3000/ml total counts	
	35	25 ml filtered	10 coliforms	
	40	25 ml filtered	7 coliforms	
		10^{-3}, 10^{-2} dilutions	3300/ml total counts	
	42	10^{-4}, 10^{-3} dilutions	30,000/ml total counts	K. pneumoniae

(Continued on next page)

Table 2-A. (Continued)

	Day	Test	Observation	Identification
Tank C	1	1 liter filtered	7 coliforms	
	3	Staves scraped	Coliforms present	
	5	1 liter filtered	No coliforms	
	7	Staves scraped	No coliforms	
	8	500 ml filtered	1000 coliforms	*E. agglomerans*
	22	100 ml filtered	3 coliforms	*K. pneumoniae* and *E. agglomerans*
	34	100 ml filtered	300 coliforms	
		10^{-3}, 10^{-2} dilutions	24,000/ml total counts	
	35	25 ml filtered	120 coliforms	
	40	25 ml filtered	150 coliforms	
		10^{-3}, 10^{-2} dilutions	35,000/ml total counts	
	42	10^{-4}, 10^{-3}, 10^{-2} dilutions	16,500/ml total counts	*K. pneumoniae*

[a]Water in tanks was Corvallis tap water with an initial chlorine concentration of 0.2-0.3 ppm. Samples were removed and water immediately replaced with an equal volume. Water was static during entire experiment. Coliform counts were made using m-Endo LES agar, total counts using nutrient agar. Isolates were identified as explained in Materials and Methods section.

examined). Presumptive tests showed these coliform isolates to be K. pneumoniae.

On day 14, the tank was drained and stave scrapings revealed heavy coliform growth within a thick slime layer. This slime was then scrubbed off using a chlorine solution of 200 ppm total concentration. The tank was refilled with Corvallis tap water. Coliform counts continued negative until day 21, when a water sample yielded 4.3 coliforms/100 ml. Again, the organisms were identified as K. pneumoniae. The tank was once again drained and refilled. By day 30, one coliform/100 ml was seen. Tests continued negative until day 50, when Klebsiellae were isolated from nutrient agar plates at the 10^{-2} and 10^{-3} dilutions.

Table 2-A also gives the history of Tanks B and C. These were tested more or less identically as they arrived at the laboratory on the same day. These tanks were filled with tap water and held for 5 days in Portland, then drained and shipped to OSU.

These two tanks were filled with Corvallis tap water containing 0.4 ppm total chlorine, and samples were removed later that same day (day 1). Both tanks gave positive coliform counts. On day 3, the tanks were drained and three staves were scraped from each tank. The scrapings of the tank wall slime (below water level) also showed the presence of coliforms. On day 6, 500-ml water samples yielded

coliform growth in both tanks. On day 22, 100-ml samples were tested. Tank B had 160 coliforms/100 ml, while Tank C showed 3/100 ml. Identification of five isolates indicated Klebsiella pneumoniae.

On the 34th day of monitoring, Tank B yielded 48 coliforms/100 ml; Tank C showed 300/100 ml. Total bacterial counts on nutrient agar showed Tank B to have 3×10^5/100 ml and Tank C, 24×10^5/100 ml. By day 40, Tank B gave counts of 28 coliforms/100 ml, while Tank C had 600 coliforms/100 ml. Counts on the 42nd day, on nutrient agar, showed 2×10^6/100 ml (Tank B) and 18×10^6/100 ml (Tank C). Identification of oxidase negative, gram negative rods from 10^{-1}, 10^{-2}, and 10^{-3} dilutions on nutrient agar revealed K. pneumoniae. Exhaustive testing of 100, 500, and 1000-ml samples of Corvallis tap water showed no coliforms, and the total chlorine concentration ranged from 0.3 to 0.6 ppm during all of these preliminary investigations. The water in these three tanks remained static at all times; any sample removed for examination was immediately replaced by an equal volume of water.

One experiment was run to compare the coliform counts obtained on m-Endo LES agar and the nature of organisms growing at higher dilutions on Bacto nutrient agar (non-selective). Table 2-B shows the results of this experiment. Equal water samples from each of the three tanks were diluted and plated on both media. Counts

Table 2-B. Comparison of viable counts of water samples using nutrient agar and m-Endo LES agar.[a]

Tank	m-Endo LES (35 C) (coliforms/100 ml)	Nutrient agar (30 C) (total counts/100 ml)
A	0	500×10^3
B	1×10^3	2500×10^3
C	0.6×10^3	3000×10^3

[a]Thirty-two colonies were randomly isolated from the nutrient agar plates (all three tanks) at the 10^{-4} and 10^{-3} dilutions. Nine colonies were oxidase-positive non-coliforms; the remaining 23 colonies were identified as Klebsiella pneumoniae using methods described in the Materials and Methods section.

were compared after suitable incubation periods, and identification made of random isolates. Total counts on nutrient agar exceeded the coliform count on the selective medium by at least 1000-fold. Of the 32 colonies picked randomly from the 10^{-2} and 10^{-3} dilutions of four nutrient agar plates, 23 were later identified as Klebsiella pneumoniae. The other nine organisms were oxidase-positive non-coliforms.

Due to the high coliform counts, Tank B was chosen for further studies in testing the response of coliforms to known chlorine amendments. The history of this tank has been previously described. It was believed that by maintaining an adequate chlorine residual within the tank water, no coliform problem would develop.

Coliform counts in the tank water were monitored daily by filtering 1, 10, 100, or 250-ml samples using a membrane filter apparatus (pore size 0.45 μm). The chlorine concentration was

checked by the OTO method. Random staves were also periodically scraped, and the resulting liquid was streaked onto m-Endo LES agar. Days 1 and 2 show counts obtained before beginning treatment. This treatment was initiated by draining the tank, and scrubbing the bottom and sides with a solution containing 1000 ppm chlorine. This was then rinsed with tap water and filled initially with water at 100 ppm total chlorine concentration (OTO method).

The results of this experiment are summarized in Figure 2-C. Due to the log nature of the graph, it was necessary to signify sampling points at the 10^{-1} level. These actual counts indicate no coliforms were detected (0/100 ml).

Prior to beginning the chlorine regimen, total coliform counts were in the range of 1400-1900/100 ml water. Immediately after the initial treatment (day 3), counts in the water and on stave surfaces were undetectable (no coliforms/250 ml filtered).

On day 4, water samples were again taken which yielded no coliforms. The tank was drained and four staves were scraped. No coliforms were detected and the tank was refilled with 50 ppm total chlorine. This process was continued and on day 6 similar samples were taken and the tank was refilled at 25 ppm total chlorine. On day 8, one coliform appeared on the filter from a 250-ml sample. No coliforms were isolated from stave scrapings.

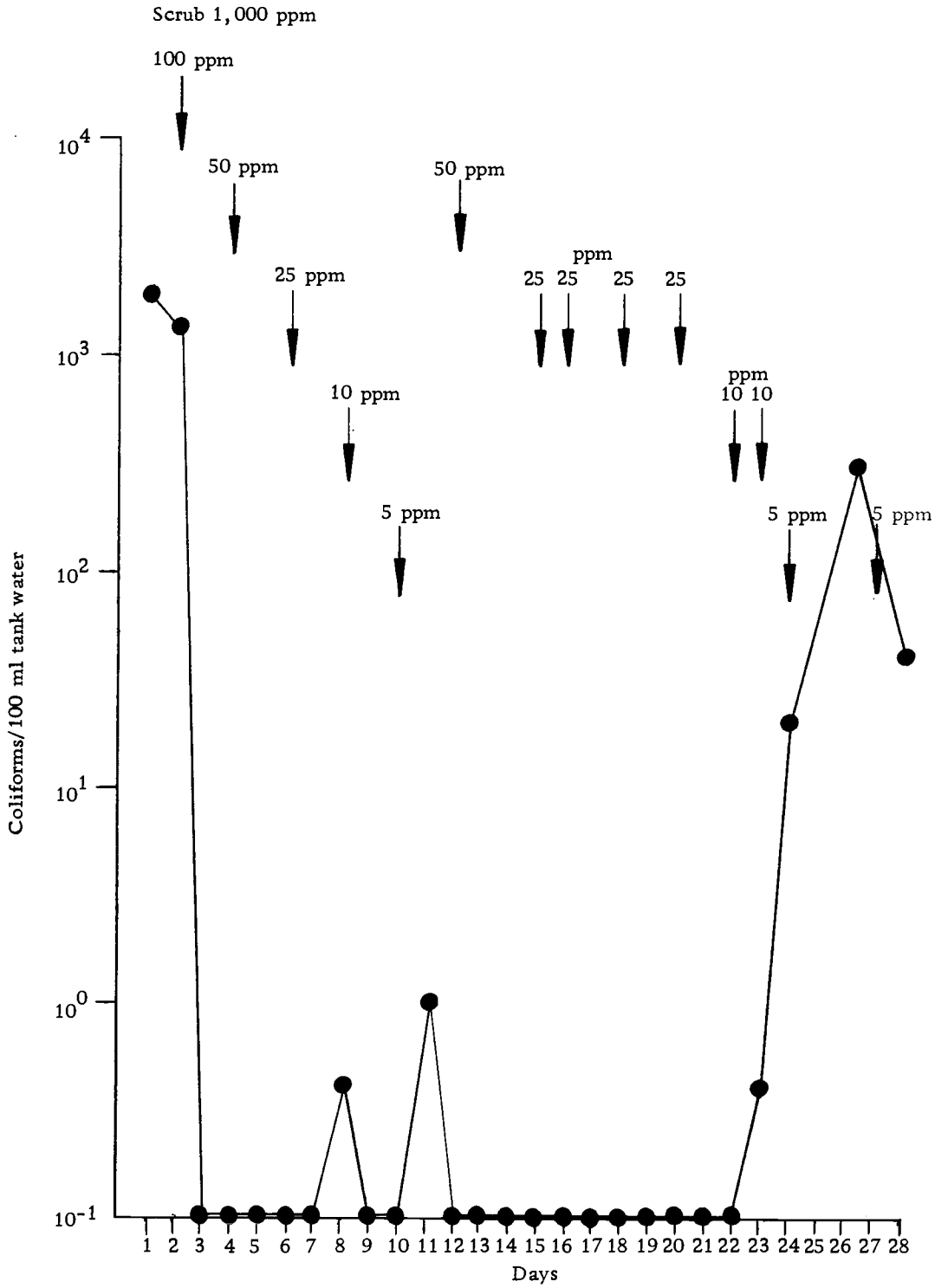

Figure 2-C. Response of coliforms in experimental redwood tank to known chlorine amendments.

The initial chlorine residual was successively decreased to 10 ppm and then to 5 ppm total chlorine. On day 11, one coliform appeared from the 100-ml water sample. As it was believed that 5-10 ppm initial chlorine residual was approaching a threshold for appearance of coliforms, the chlorine concentration was increased back to 50 ppm on day 12.

The increase in chlorine immediately controlled the coliform numbers as did the subsequent changes to 25 ppm on day 22. The second regimen at 10 and 5 ppm resulted in the reappearance of coliforms on days 23-28. This experiment was terminated after 30 days.

The appearance of coliforms in the tank water with initial chlorine amendments of 5-10 ppm raised the question of an unusual resistance to chlorination. As a result, an experiment was devised to determine the chlorine sensitivity of Klebsiella organisms, using Corvallis tap water as a source of chlorine. Seven K. pneumoniae isolates were used, encompassing both environmental and clinical types. Five isolates (BW 3, PC 4, K 2, SL 3, and PC 2) came from redwood tanks in the field studies described earlier (Chapter I), while the other two were clinical isolates obtained from patients having hospital-acquired infections. (UOMS: University of Oregon Medical School; MUSC: Medical University of South Carolina.)

The isolates were grown in nutrient broth for 20 $\pm$ 1 hr at 35 C; this was assumed to result in a population density of 10^9 organisms/ml.

They were diluted to the desired concentration using sterile distilled water (generally 150-350 organisms/10 ml). The cells were then exposed to either Corvallis tap water (0.4-0.7 ppm chlorine residual) or sterile distilled water (no chlorine residual). After 0, 30, and 60 min exposure times, 10 ml samples were filtered and placed onto m-Endo LES agar for viable counts (35 C for 24 hr).

Table 2-C shows the results of this experiment. In no instance was any coliform growth seen when the organisms were exposed to Corvallis tap water. Zero time samples were plated within 60-90 sec of initial exposure time. Using sterile distilled water, little if any significant changes in counts were observed.

A set of experiments was then performed which examined the influence of a polyethylene liner on loss of chlorine in Tank B. Table 2-D summarizes the results. In the initial experiments without any liner, chlorine was added as a concentrated stock solution and diluted out as the tank was filled. Experiment 1 (without liner) demonstrates a decrease from an initial chlorine concentration of 4.0 ppm to 0.5 ppm in 2 days, while Experiment 2 (without liner) shows a complete loss of detectable chlorine (from 4.0 ppm) in 4 days.

The second set of experiments involved the use of the plastic liner sent from National Tank and Pipe. Experiment 1 (with liner) started with tap water being added to the tank with a measured chlorine concentration of 0.6 ppm; after 10 days the residual was 0.1 ppm.

Table 2-C. Comparative viability of *K. pneumoniae* isolates exposed to chlorinated Corvallis drinking water and distilled water.[a]

Isolate	Time of exposure (min)	Counts/10 ml sample	
		Tap water	Distilled water
BW 3	0	No growth	255
	30	No growth	240
	60	No growth	270
PC 4	0	No growth	350
	30	No growth	305
	60	No growth	295
K 2	0	No growth	200
	30	No growth	200
	60	No growth	210
UOMS 1[b]	0	No growth	150
	30	No growth	140
	60	No growth	120
MUSC 2[b]	0	No growth	8
	30	No growth	10
	60	No growth	12
SL 3	0	No growth	210
	30	No growth	130
	60	No growth	155
PC 2	0	No growth	240
	30	No growth	140
	60	No growth	205

[a]Cells were diluted to final concentration of 150-250/10 ml and plated on m-Endo LES agar. Counts were made after 24 hr.

[b]UOMS - University of Oregon Medical School; MUSC - Medical University of South Carolina.

Table 2-D. Influence of polyethylene liner on loss of chlorine from an experimental redwood tank.

With liner		Without liner	
Day	Cl residual	Day	Cl residual
	Experiment 1		
0	0.6	0	4.0
1	0.5	0.125	3.5
2	0.4	0.85	2.5
3	0.35	1.25	2.5
5	0.2	2.0	0.5
6	0.2		
7	0.1		
8	0.1		
10	0.1		
	Experiment 2		
0	0.7	0	4.0
2	0.6	4	0
10	0.4		
11	0.4		
12	0.4		
14	0.35		
17	0.3		
18	0.3		
19	0.3		
20	0.25		
24	0.1		
25	0.1		
26	0.1		
28	0.1		
31	0.1		
35	0.05		
40	0		

Experiment 2 (with liner) ran over a 40 day period, and showed a decrease from an initial residual of 0.7 ppm to 0.0 ppm on the 40th day when the experiment was terminated.

It is apparent from these observations that chlorine is not escaping through the lid, but is instead related to the demand exerted by the presence of materials in the tank structure. In a separate series of experiments, the response of an experimental laboratory tank to a circulating chlorinated water supply was examined. Tank B was altered to provide an inlet and outlet; the outlet being located $1\frac{1}{2}$ inches from the top of the tank, while the inlet was one inch from the bottom. Connecting rubber hoses brought a tap water supply through the lower spigot, and the drainage from the upper tank valve flowed into a nearby sink. All experimental samples and estimates of water flow were taken from the overflow hose. Figure 2-B shows the modifications made in Tank B for this experiment.

The history of Tank B has been described elsewhere. To attempt an initial sterilization and simultaneous removal of surface organic matter from the tank interior, a concentrated soda ash solution (2 lb/100 gal) was left in the tank for 48 hr. Extensive leaching of material was evidenced by a dark red coloration of water when drained from the tank. A solution of 200 ppm total chlorine (using Hth dry granular chlorine) was then placed in the tank for 24 hr.

The tank was then connected to the tap water spigot and circulation began. Over the 41 day duration of this experiment, the turnover time ranged from 16-46% tank capacity/hr, with an average of 20%/hr.

The incoming chlorine residual ranged from 0.3-0.6 ppm and averaged 0.47 ppm, while the overflow residual ranged from 0.5-0.0, and averaged 0.2 ppm.

Periodic examinations of the water consisted of filtering 10, 50, and 100-ml samples using membrane filtration and incubating filters on m-Endo LES agar for total coliform counts. Selected stayes were also periodically scraped and qualitatively examined for coliforms using m-Endo LES as the plating medium.

The results given in Table 2-E show the progression of this experiment. At no time were any coliforms seen in either the water or on the staves. No slime was seen to develop on staves. A detectable chlorine residual was maintained for most of the experiment (only two instances of no residual: days 5 and 39). At the end of 41 days, the experiment was terminated.

Discussion

The main purpose of the described experiments on small redwood tanks was to provide data on the appearance and development of coliforms, and to clarify the extent and nature of the chlorine demand of wooden tanks. Using small laboratory tanks would provide a very accessible unit for study, and provide conditions similar to those in the field tanks.

Table 2-E. Influence of circulating chlorinated water supply on Tank B.[a]

Day	Flow rate (% capacity/hr)	Chlorine in (ppm)	Chlorine out (ppm)	Water quality
1	46	0.6	0.4	
2	23	0.6	0.5	
3	16	-	-	
4	28	0.4	0.15	
5	23	0.5	0.05	No coliforms
8	23	0.5	0.2	
9	23	0.4	0.2	
10	23	0.4	0.1	
11	23	0.4	0.1	
13	23	0.3	0.1	No coliforms
16	23	0.5	0.2	
17	18	0.3	0.15	No coliforms
18	18	0.5	0.2	
19	23	0.5	0.1	
20	23	0.5	0.2	No coliforms
23	18	0.6	0.2	
24	18	0.4	0.2	
25	23	0.5	0.2	No coliforms
26	18	0.5	0.2	
30	18	0.45	0.3	
31	18	0.4	0.25	No coliforms
32	18	0.4	0.1	
33	18	0.5	0.2	
38	23	0.6	0.5	
39	18	0.4	0.0	No coliforms
40	18	0.6	0.2	
41	18	0.5	0.1	
Range:	16-46	0.3-0.6	0.0-0.5	
Average:	20	0.47	0.2	

[a]The tank was initially treated with a soda ash solution at 2 lb/1000 gal. This remained in the tank for 48 hr. The water was drained and replaced with a solution containing 200 ppm total chlorine and incubated for 24 hr. Water quality examinations consisted of membrane filtration of 10, 50, and 100 ml samples. Filters were incubated on m-Endo LES agar at 35 C for 24 hr. Staves were scraped with sterile glass slides, the material diluted and streaked onto m-Endo LES.

The studies on the three small tanks were significant in that they confirmed the patterns previously seen in field tanks. Coliform organisms first appeared in relatively small numbers on the staves of the tank interiors below the water line. This was almost always accompanied by the development of a slime layer. After a short period of time, the organisms were found in the water in increasingly larger numbers. As extensive testing of the incoming water revealed no coliforms present, it seems apparent that the coliforms arise from the wood interior and were able to multiply using an unknown growth factor(s) within the tank environment.

One sidelight of these observations was given in the experiment involving differential counts on nutrient agar and m-Endo LES agar. The selective m-Endo LES medium, while providing the widely accepted system for the rapid isolation and enumeration of coliforms, also gives a lower estimate of the numbers present due to its highly selective nature. Using a non-selective general purpose medium such as nutrient agar showed actual coliform counts to be much higher (1000-fold). However, m-Endo LES is still the medium of choice for coliform isolation due to the rapid ease in enumeration of these organisms directly from aquatic environments.

The fact that chlorine amendments can effectively limit the appearance of coliforms was shown in the experiment involving Tank B. If a relatively high (25-50 ppm) initial chlorine concentration was

added to the tank, no coliforms appeared in the water when examined. At the threshold of 5-10 ppm initial chlorine, coliforms were again seen in high numbers. The threshold seems high when compared to the 0.4-0.6 ppm chlorine concentration seen in municipal water systems, but these tests involved an uncirculating body of water that lost chlorine residual rapidly after addition. Although difficult to test with the present techniques, there is very likely a chlorine gradient which probably approaches zero on the stave surfaces.

This rapid loss of detectable chlorine in the tank water was shown by some simple observations using Tank B. A residual of 4.0 ppm total chlorine was lost in 4 days in a static water situation. This was due to high chlorine demand by the wood, and its release of organic matter into the water.

The use of a polyethylene liner to eliminate the chlorine demand of the wood showed that very little chlorine was lost through the water-air interface. When the demand of the wood was eliminated, a relatively low residual of 0.7 ppm could be maintained (with slow decline to 0.0 ppm) over nearly 40 days.

To examine the possibility that the genus Klebsiella might possess a special resistance to chlorine, an experiment was performed using tap water as the source of chlorine. The results showed immediate loss of viability when cell suspensions were exposed to the

chlorine contained in a municipal water supply (0.4-0.7 ppm). Hence, if a suitable residual can be sustained in a redwood tank system, coliform loss should be complete.

The last experiment attempted to simulate a large tank system having a specific turnover time and regular chlorine amendments. The results are general in nature but nevertheless serve to indicate the need for careful monitoring of chlorine concentration and an accompanying water turnover to distribute the chlorine in the tank water. In this experiment, it was seen that circulation kept the residual at a detectable level throughout the tank and prevented any development of coliforms on staves or in the tank water.

The work on these three small tanks provided many insights into the coliform problems as seen in the larger tank systems. With no water circulation or chlorine amendments, coliforms will soon develop on the staves and then move to the water. Even with a completely coliform-free source of water, the organisms will appear and increase in numbers, apparently being sustained by a sufficient supply of growth factors emanating from the wood itself. Since the incoming water was extensively monitored and shown to be free from coliforms, these bacteria probably gained entrance from the contaminated redwood.

When a suitable chlorine residual (0.2 ppm) can be maintained throughout the tank, Klebsiellae will not be able to survive and the

coliform problem should never develop. Sustaining an adequate residual, however, necessitates a constant monitoring arrangement with provisions made for varying turnover times and needed chlorine amendments. The latter are a function of the tank size and amount of water consumed daily from such reservoirs.

EXAMINATION OF REDWOOD AS A SOURCE OF COLIFORM ORGANISMS

Abstract

In these experiments redwood was examined as the source of coliform organisms found in water tank systems. Liquid expressed from redwood blocks yielded coliforms as did both sawdust and wood chips when incubated in suitable liquid enrichment medium. Total bacterial counts in the expressed liquid often exceeded 10^5 cells/ml, while coliforms (when present) exceeded 10^2/ml. Contact plates of freshly cut wood surfaces revealed high coliform populations within the wood. Klebsiella and Enterobacter were found in fresh wood samples, while only Enterobacter persisted in older samples. At these high cell densities, coliform organisms must be capable of growth within the wood utilizing nutrient materials contained within the wood structure.

Materials and Methods

In an attempt to isolate coliform organisms directly from redwood, a number of different procedures were investigated. These included enrichment experiments with wood chips, sawdust, and redwood blocks, as well as direct enumeration trials.

Using sawdust from a given wood sample, a pre-weighed amount (40 g) was incubated in sterile distilled water in a 1-liter flask (one shaken, one static, both at room temperature). The liquid was examined periodically for pH, optical density, and presence of coliforms. Samples of liquid were periodically removed and filtered using membrane filters (pore size 0.45 μm). These were incubated on m-Endo LES agar at 35 C for 24 hr. For total counts, dilutions were made using sterile distilled water, and spread plates were prepared using Bacto nutrient agar. These were incubated at 30 C for 24 to 48 hr.

A number of experiments involved subjecting small wood block samples to high pressure in an effort to isolate coliforms from the expressed liquid. Blocks of wood with all outer surfaces removed were placed in a Carter press and the liquid expressed at 12,000-15,000 psi. The liquid was collected on a piece of sterile foil beneath each sample. Polyvinyl pyrolidone (PVP) was used in most samples to neutralize phenolic compounds released as the wood structure was

destroyed. It was generally made up as a stock solution (1:10 w/v) with sterile distilled water, and applied directly to the collecting foil and/or used in the dilution blanks. Most diluent used was made of 0.01 M Tris buffer (pH 7.0), although in some cases sterile distilled water was employed. Samples removed from the collecting foil were either plated directly, or diluted and then plated. Bacto nutrient agar was used for total bacterial counts, Bacto m-Endo LES agar for total coliforms, and Bacto mFC agar for fecal coliforms. These were incubated at 30, 35, and 44.5 C respectively, and counts were made after 24 and 48 hr.

Another experiment involved removing the outer surfaces of redwood blocks and successively cutting 3/8 inch sections through each block. Each freshly cut surface was brought into contact with the surface of nutrient agar, m-Endo LES agar, and/or mFC agar plates. These plates were incubated at the indicated temperatures, and counts recorded after 24 and 48 hr. The rotary saw blade used to prepare the sections and the saw bench top were swabbed with 70% ethanol between each cut, and then surface swabbed and spread onto m-Endo LES agar to check for sterility. These plates were incubated at 35 C for 48 hr.

Wood chips from various samples were also examined. The chips were pre-weighed, then incubated in 50 to 250 ml of 0.01 M Tris buffer on a shaker at 35 C for 15 min. The liquid being sampled was

diluted in sterile Tris and plated on m-Endo LES and nutrient agars using the spread plate technique.

Enrichment experiments were performed using a modified mFC broth without added rosalic acid. One gram of redwood sawdust or chips was incubated in 10 ml mFC broth for 3 days at 37 C, the liquid was then streaked (one loopful) onto m-Endo LES agar (incubated at 37 C) and mFC agar (incubated at 44.5 C). Coliform colonies were purified and maintained on nutrient agar slants at 5 C for later species identification.

In all experiments outlined above, coliform isolates were inoculated into Bacto TSI agar slants to check for fermentation, H_2S formation, and gas production from lactose, sucrose, and glucose. These were incubated at 35 C for 48 hr. The presence of lysine and ornithine decarboxylase and arginine dihydrolase activities was checked using Bacto Moellers broth base and the appropriate amino acid. These were incubated at 35 C and read each day for 4 days. All Enterobacter and Klebsiella isolates were streaked onto nutrient agar slants and maintained at 5 C until further taxonomic studies could be performed. Any yeast or mold isolates were purified using either Bacto potato dextrose agar or Bacto malt agar, then stored on malt agar slants at room temperature until identification could be arranged.

Results

Many procedures were utilized with varying degrees of success in an attempt to isolate coliform organisms directly from redwood. These experiments involved the examination of sawdust, wood chips, entire blocks of wood, and liquid expressed from the wood interior.

Table 3-A shows the results of an experiment involving the enumeration of total bacteria and coliforms in redwood sawdust. These samples were from both heartwood and sapwood, the sawdust being collected as aseptically as possible from beneath a power saw. Sawdust was added to each of two flasks. One was incubated statically and the other on a shaker. Both were incubated at room temperature. These enrichments were first examined 7 days after inoculation.

The pH of the extract in the shaken flask was 4.5 while the optical density was 1.20 (550 nm) on day 7. The extract in the static flask had a pH of 6.0 and an optical density of 0.26. Fluid samples processed on day 7 showed confluent coliform growth per 10 ml filtered samples. Total bacterial counts on nutrient agar showed 500×10^3/ml from the static vessel and 200×10^3/ml from the shaken one.

On day 11, there were little variations in pH but bacterial counts had increased dramatically. Coliform counts on m-Endo LES agar were higher for the shaken sample; 2500/ml compared to

Table 3-A. Isolation of coliform organisms from redwood sawdust samples.[a]

Day	Shaken	Static
7	pH = 4.5	pH = 6.0
	Optical density = 1.20	Optical density = 0.26
	TNTC/10 ml sample filtered (m-Endo LES agar)	TNTC/10 ml sample filtered (m-Endo LES agar)
	2.0×10^5/ml (nutrient agar)	5.0×10^5/ml (nutrient agar)
11	pH = 5.0	pH = 6.0
	2.5×10^3/ml (m-Endo LES agar)	7.5×10^2/ml (m-Endo LES agar)
	4.0×10^7/ml (nutrient agar)	3.0×10^7/ml (nutrient agar)
14	2.0×10^6/ml (m-Endo LES agar)	2.2×10^6/ml (m-Endo LES agar)
20	4.2×10^4/ml (m-Endo LES agar)	7.0×10^5/ml (m-Endo LES agar)
21	2.5×10^8/ml (nutrient agar)	2.5×10^8/ml (nutrient agar)

[a]Each flask contained 40 g of redwood sawdust plus 1 liter sterile distilled water. One flask was incubated statically, the other on a shaker, both at room temperature. Dilutions from 10^{-1} to 10^{-6} were plated on both nutrient agar and m-Endo LES agar. The nutrient agar plates were incubated at 30 C, the m-Endo LES agar plates at 35 C. Counts were made at 24 hr.

750/ml for the static sample and total counts on nutrient agar were 400 x 10^5/ml and 30 x 10^5/ml respectively. At the end of 14 days, however, coliform counts from the static flask were higher: 225 x 10^4/ml as compared to 2 x 10^4/ml in the shaken flask.

By the 21st day, when the experiment was terminated, coliform counts were again higher in the static sample: 70 x 10^4/ml, while the shaken sample gave 4.2 x 10^4/ml. The total bacterial counts were virtually identical at 250 x 10^6/ml.

Of ten coliform organisms picked at random (five from the shaken flask, five from the static flask) and inoculated into TSI agar slants, three did not grow while seven were fermentative and three of the seven were aerogenic. These seven fermentative organisms were identified as Enterobacter agglomerans (5), Enterobacter cloacae (1), and Enterobacter aerogenes (1).

A total of four separate studies examined the bacterial and coliform content of liquid expressed from the wood interior. Tables 3-B and 3-C present the results of these experiments. The first experiment was carried out at the National Tank and Pipe plant in Portland, using the facilities of their chemical research laboratory. Finished wood samples (collected from storage facilities and destined for redwood water tanks) were placed on sterile foil and subjected to approximately 15,000 psi using a Carter press. The expressed liquid was collected as quickly as possible using a sterile pipette, and

Table 3-B. Isolation of coliform organisms from liquid expressed from redwood.

Type of wood	Counts x 10^3, nutrient agar at 30 C for 48 hr
Experiment 1 (National Tank, Portland)[a]	
Green weathered heartwood	5, 10, 15 (3 samples + PVP)
Construction heartwood	2, 10, 15 (3 samples + PVP); 40 (1 sample - PVP)
Air seasoned heartwood	NG, 2, 10, 10, TNTC, TNTC (6 samples + PVP); NG (1 sample - PVP)

	Experiment 2 (OSU, Corvallis)[b]	
	Counts x 10^3, nutrient agar at 30 C for 48 hr	Counts x 10^2, m-Endo LES agar at 35 C for 48 hr
Sinker heartwood	250, 400 (2 samples + PVP) 150, 300 (2 samples - PVP)	No growth at any dilution (2 samples + PVP)
Wide ring count heartwood	3000, 6000 (2 samples with PVP on collecting foil only)	TNTC (2 samples + PVP)
Heavy stain heartwood	2 (1 sample - PVP) 0.5 (1 sample + PVP)	Not done
Heavy sapwood	400, 3000 (2 samples with PVP on collecting foil)	20 (1 sample + PVP)
Young growth heartwood	NG, NG (2 samples + PVP) NG (1 sample - PVP)	NG at any dilution (1 sample + PVP)

(Continued on next page)

Table 3-B. (Continued)

Type of wood	Counts x 10^3, m-Endo LES for 48 hr (30 C)	(35 C)	(40 C)
	Experiment 3 (OSU, Corvallis)[c]		
Sinker heartwood	1 (1)	NG (4)	NG (4)
Heavy sapwood	20-50 (3)	5-50 (4)	Not done
Wide ring count sapwood	2-500 (5)	NG (1)	NG (2)
Young growth heartwood	0-.03 (2)	NG (1)	NG (2)
	Counts x 10^3, nutrient agar at 30 C for 48 hr		
Sinker heartwood	13-500 (6)		
Heavy sapwood	10-500 (6)		
Wide ring count sapwood	13-500 (4)		
Young growth heartwood	30-88 (4)		

[a]Dilution ranged from 10^{-1} to 10^{-4} on all samples. +PVP indicates the use of polyvinyl pyrolidone as part of the diluent, while -PVP indicates the absence of this compound. TNTC means uncountable confluent growth. NG indicates no growth.

[b]Dilutions ranged from 10^{-1} to 10^{-4} for all samples tested. Samples were processed on both nutrient agar at 30 C and m-Endo LES agar at 35 C.

[c]Dilutions were made from 10^0 to 10^4 for each sample. Samples were processed on both nutrient agar at 30 C and m-Endo LES agar at 35 C. All samples were processed with polyvinyl pyrolidone as part of the diluent. Numbers in parentheses indicate the number of different samples processed for each type of wood.

Table 3-C. Isolation of coliform organisms from California mill redwood samples: expressed liquid from small blocks.[a]

Wood sample	Counts/ml on three media		
	Nutrient agar (37 C)	m-Endo LES (37 C)	mFC (44.5 C)
CT 3	5.2×10^4 (1)	5.0×10^2 (1)	No growth (1)
CT 4	TNTC, 3.0×10^4 (2)	NG, 1.0×10^4 (2)	No growth (2)
CT 5	5.0×10^4 (1)	6.0×10^3 (1)	No growth (1)
CT 6	1.2, 1.3×10^5 (2)	0.3, 1.5×10^6 (2)	No growth (2)
CT 7	1000×10^3 (1)	3.0, 4.0×10^5 (2)	No growth (2)
CT 8	No growth (1)	No growth (1)	No growth (1)
CT 9	1.2×10^5 (1)	TNTC at 10^{-4} (1)	2.0×10^2 non-fecal coliforms (1)

[a]Wood blocks averaged 2 in. x 3 in. A stock solution of polyvinyl pyrolidone (1:10 w/v with sterile distilled water) was used at 1 ml per dilution blank. Diluent was 0.01 M Tris buffer (pH 7). Also, 2 ml of the PVP stock was placed directly on collecting foil. Dilutions from 10^{-1} to 10^{-3} were made and plated on both nutrient agar and m-Endo LES agar. These media were incubated at 37 C to enhance selectivity for Klebsiella organisms. Direct samples of 0.1 ml were plated on mFC agar and incubated at 44.5 C. Numbers in parentheses indicate number of samples examined for each type of wood. TNTC means uncountable confluent growth. NG means no growth. Source of wood samples is as follows: CT 3 - trim green heartwood/Korbel mill; CT 4 - heartwood/sorter/Korbel mill; CT 5 - heartwood and sapwood/sorter/Korbel mill; CT 6 - heartwood and sapwood/chip conveyer/Korbel mill; CT 7 - heartwood flat grain and sapwood clear grain/sorter/Korbel mill; CT 8 - sapwood/sorter/Korbel mill; CT 9 - heartwood and sapwood/wastewood pile/Korbel mill.

diluted through sterile water blanks containing 1 ml of PVP stock (1:10 w/v with sterile distilled water) or plated directly without dilution. A second group of samples was diluted in sterile water blanks without PVP.

The results of this extraction gave no coliform growth on the m-Endo LES agar media (24 or 48 hr incubation). Small particles of the PVP may have interfered with the recognition of small coliform colonies and there was also a high incidence of mold growth covering many plates. The total bacterial counts on nutrient agar showed a wide range in values for the different wood samples. Some 75 isolates from the nutrient agar plates were tested for fermentative abilities on TSI agar slants. Those showing any degree of fermentation (about 40%) were all shown to be yeasts upon microscopic examination. Twenty-two mold isolates from this experiment were retained on malt agar slants at room temperature for later identification.

The second extraction experiment was performed at Oregon State University. Coliforms were obtained from the wide ring count sapwood (WRC) and heavy sapwood (HS) samples. WRC had confluent growth of coliforms on all plates, while HS had 2000 coliforms/ml. Liquid expressed from the young growth heartwood (YGH) sample contained no microbes capable of growth on the two media used. Species identification of coliform organisms was not attempted in these experiments.

The third experiment (Table 3-B) also run at the Oregon State laboratory, was basically similar to the preceding ones. Changes were made in the processing of selective coliform media in triplicate with incubation at three different temperatures; 30, 35, and 40 C. In addition, all wood blocks were flooded with 2 ml of PVP (1:5 w/v) as soon as the liquid was expressed from within the block. The same wood material was used as in Experiment 2 (Table 3-B) to prepare the smaller blocks.

Definite effects of the three incubation temperatures can be seen. No sample gave growth on m-Endo LES agar at 40 C, one yielded colonies at 35 C, and all gave green sheen colonies at 30 C. There was a wide range of total counts for all samples on nutrient agar at 30 C. Identification of some 20 coliform isolates from the HSW and WRS samples yielded Enterobacter agglomerans.

Table 3-C summarizes the results of a similar extraction experiment carried out using fresh redwood samples obtained from the Korbel mill in Arcata, California. Samples were processed in a similar manner to earlier experiments, using Tris buffer as diluent and PVP stock flooding each wood sample as liquid was expressed. Seven samples were examined and three isolation media were used: nutrient agar, m-Endo Les agar, and mFC agar. In this experiment, however, both the nutrient agar and m-Endo LES agar were incubated

at 37 C to enrich for the growth of Klebsiella organisms (4). mFC agar was incubated at 44.5 C for growth of fecal coliforms.

Of the seven samples, only one produced no growth on any of the media used (CT 8). On mFC agar, only one sample had growth (CT 9); this was 200 pink colonies/ml which are defined as non-fecal coliforms. All samples had growth on both nutrient agar and m-Endo LES agar, although the counts were generally higher on the non-selective nutrient agar medium.

Forty-five isolates from both nutrient agar and m-Endo LES agar plates were identified. Presumptive coliforms (green sheen on m-Endo LES medium) were inoculated into media for detection of lysine and ornithine decarboxylase activities. Of the 45 isolates examined, 6 were Klebsiella pneumoniae, 48 were Enterobacter spp., and 1 did not grow.

Another set of experiments involving isolation of coliform organisms from redwood consisted of direct contact of wood surfaces to the surface of an agar plate. The results are given in Table 3-D. Two wood samples that had yielded coliforms in the expressed liquid experiments were used (HSW and WRC).

First, the outer surfaces of small blocks ($2\frac{1}{2}$ x 2 in.) were prepared as aseptically as possible using a power saw whose blade had been sterilized with 95% ethanol. The outer surfaces of the wood block were touched to three m-Endo LES agar plates, and these were

Table 3-D. Isolation of coliform organisms from freshly cut wood surfaces.

Type of wood	Area tested	Incubation temperature (C)	Counts on m-Endo LES agar
	Experiment 1 - Outer surface of wood blocks[a]		
Heavy sapwood	Outer surface ($2\frac{1}{2}$ x 2 in.)	30 C	Greater than 1000 (2)
		35 C	Greater than 1000 (2)
		40 C	No growth (1), 11 coliforms (1)
Wide ring count sapwood	Outer surface ($2\frac{1}{2}$ x $2\frac{1}{2}$ in.)	30 C	Greater than 1000 (2)
		35 C	Greater than 1000 (2)
		40 C	No growth (2)

Experiment 2 - Serial cuts through wood blocks $1\frac{1}{2}$ x $1\frac{1}{2}$ x $1\frac{1}{2}$ in.[b]

		Counts on m-Endo LES agar (30 C)	Counts on m-Endo LES agar (35 C)
Heavy sapwood	Upper surface	TNTC coliforms	TNTC coliforms
	2	TNTC coliforms	12 coliforms
	3	TNTC non-coliforms	10 coliforms
	4	TNTC coliforms	No growth
	Inner surface	50 non-coliforms	No growth
	6	TNTC coliforms	No growth

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Table 3-D. (Continued)

Type of wood	Area tested	Counts on m-Endo LES agar (30 C)	Counts on m-Endo LES agar (35 C)
	7	TNTC non-coliforms	15 coliforms
	8	TNTC non-coliforms	30 coliforms
	Lower surface	TNTC coliforms	TNTC coliforms
Wide ring count sapwood	Upper surface	TNTC coliforms	TNTC coliforms
	2	TNTC coliforms	TNTC coliforms
	Inner surface	TNTC coliforms	TNTC coliforms
	4	TNTC coliforms	TNTC coliforms
	5	TNTC coliforms	TNTC coliforms
	Lower surface	TNTC coliforms	TNTC coliforms

[a]Wood surfaces were touched to the agar surface of the m-Endo LES agar medium. Numbers in parentheses indicate the number of samples tested.

[b]Blocks were cut serially throughout, each cut of approximately 3/8 in. Each freshly cut surface was touched to surface of m-Endo LES agar plate. TNTC indicates confluent uncountable growth. Saw blade was surface sterilized with 70% ethanol between each cut.

incubated at 30, 35, and 40 C. Both samples gave high counts at 30 C and 35 C, while only the HSW sample produced any growth at 40 C (11 colonies).

Next, blocks of wood ($1\frac{1}{2}$ x $1\frac{1}{2}$ in.) were serially cut into six to nine sections, each section then being pressed onto the surface of an m-Endo LES agar plate. These were done in duplicate and incubated at 30 and 35 C. Both samples yielded high numbers of coliforms located within the wood structure. Some 20 coliform isolates from both samples were identified as Enterobacter agglomerans.

Other experiments were devised in an attempt to enumerate coliforms present on wood chips and shavings. The results of these are summarized in Tables 3-E and 3-F. In the first experiment, redwood shavings were incubated in 250 ml of 0.01 Tris buffer (pH 7.0) on a shaker at 35 C for 15 min to wash off the indigenous bacterial flora. Samples were immediately plated onto either m-Endo LES agar or nutrient agar. Both media were incubated at 30 C. Two samples (WRC and HSW) showed coliform growth at greater than 1000 colonies/ml. This is equal to some 25 x 10^3/g of wood chips. These same two samples had similar counts on nutrient agar. Two other samples (HS and SH) had growth on nutrient agar but none on m-Endo LES agar.

A second experiment using wood chips produced growth from all samples. This is also summarized in Table 3-E. Chip samples

Table 3-E. Isolation of coliform organisms from redwood chips.

Type of wood	Sample weight (g)	Coliform counts/ml on m-Endo LES (30 C)	(35 C)	(40 C)
		Experiment 1[a]		
Heavy sapwood	5.16	2.5×10^5	2.0×10^5	4
Heavy stain heartwood	13.02	60	50	No growth
Young growth heartwood	14.04	1×10^4	3×10^3	No growth
Wide ring count heartwood	18.16	1×10^6	7×10^5	2
Sinker heartwood	15.92	1×10^4	1×10^4	No growth

	Coliform counts/g of wood sample at 35 C
Heavy sapwood	388
Heavy stain heartwood	3.8
Young growth heartwood	213
Wide ring count heartwood	38,540
Sinker heartwood	628

Experiment 2[b]

		Counts/ml on two media at 30 C	
		m-Endo LES	Nutrient agar
Heavy sapwood	25.90	TNTC coliforms	TNTC
Heavy stain heartwood	1.64	No growth	1×10^4

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Table 3-E. (Continued)

Type of wood	Sample weight (g)	Counts/ml on two media at 30 C	
		m-Endo LES	Nutrient agar
Young growth heartwood	2.05	No growth	No growth
Wide ring count heartwood	19.25	TNTC coliforms	TNTC
Sinker heartwood	15.32	No growth	3×10^4

[a]Chips were incubated in 50 ml of 0.01 M Tris buffer (pH 7.0) on a shaker at 35 C for 15 min. Duplicate sets of spread plates were made using dilutions from 10^{-1} to 10^{-4} and incubated at 30 and 35 C. Direct samples of 0.5 ml were plated and incubated at 40 C. All dilution blanks contained Tris as diluent.

[b]Chips were incubated in 250 ml of 0.01 M Tris buffer (pH 7.0) on a shaker at 35 C for 15 min. Direct samples of 0.1 and 0.5 ml were plated on m-Endo LES agar. Dilutions of 10^{-1} and 10^{-2} were plated on nutrient agar. Both media were incubated at 30 C for 24 hr. Dilution blanks contained Tris as diluent. TNTC means uncountable confluent growth.

Table 3-F. Isolation of coliform organisms from California mill redwood samples: Coliform enrichment using redwood chips and sawdust.[a]

Wood sample	Source	Coliforms isolated
C	Green chips from plywood mill	Klebsiella pneumoniae
D	Sawdust by boiler, plywood mill	Enterobacter spp.
E	Wet sawdust pile by shaker, Korbel mill	Klebsiella pneumoniae
F	Mixed redwood types, small chipper, Korbel mill	Klebsiella pneumoniae, Enterobacter spp.

[a] A modified mFC broth was used that contained no rosalic acid. One gram of wood sample was inoculated into 10 ml of the broth and incubated at 37 C for 3 days. One loopful of broth was then streaked on both m-Endo LES agar and mFC agar. These plates were incubated at 37 C and 44.5 C respectively.

were incubated in 50 ml of Tris buffer as before and incubated on a shaker at 35 C for 15 min. Samples were processed immediately onto m-Endo LES agar and replicate sets of plates incubated at 30, 35, and 40 C. Two samples (HSW and WRC) had growth at 40 C, while all samples had varying amounts of growth at 35 and 30 C. On three of the samples, however, coliform colonies did not appear until after 48 hr of incubation and were pinpoint in size. Green sheen colonies picked at random from all samples were found to be either yeast or Enterobacter agglomerans.

Wood chip and sawdust samples from the Korbel mill in California were examined using an enrichment in mFC broth without rosalic acid. One gram of wood sample was added to 10 ml of broth and incubated at 37 C for 3 days. A broth sample was then streaked onto mFC agar (44.5 C), and m-Endo LES agar (37 C). Table 3-F gives the results of this experiment. The D samples yielded only Enterobacter spp., the C and E samples Klebsiella pneumoniae, and the F sample both Klebsiella and Enterobacter isolates. These isolates were retained for later taxonomic studies.

Discussion

The purpose of the experiments described in this chapter was to isolate coliform organisms (particularly, Klebsiella pneumoniae) from redwood itself. The wood was examined in a number of different

forms: sawdust, wood chips, freshly cut surfaces, and liquid extracts. Inherent in these attempts at isolation was the assumption that Klebsiellae organisms are present in the actual redwood used to construct the water storage tanks. There seems to be no other source to explain their appearance in such water systems.

The association of coliform organisms with the forest environment has been documented previously in the literature. Aho et al. examined nitrogen fixing bacteria associated with fungal decay in living white fir trees (2). Isolates of both Klebsiella pneumoniae and Enterobacter spp. were found as part of this flora. Duncan and Razzell examined the bacterial populations present in bark and needle samples from hemlock, grand fir, Douglas-fir, and cedar (6). In this instance also, isolates of both Klebsiella and Enterobacter were seen.

In this study, sawdust was the first form of redwood to be examined. When samples were incubated for long periods of time, the release of phenolic compounds from the wood lowered the pH. The release of these compounds and subsequent pH drop would be expected to be inhibitory to many microorganisms. Nevertheless, even at a low pH of 5 there was still extensive bacterial growth recorded (Table 3-A). These coliforms were found to be Enterobacter spp.

Liquid extraction from redwood blocks presented the same problems of phenolic compounds being released under pressure. As the structural integrity of the wood cells and fibers was destroyed, these compounds were immediately released. The use of polyvinyl pyrolidone and Tris buffer in the preparation of the samples served to prevent reduction of viable counts by neutralizing the phenolic materials.

In these extraction experiments, the temperature of incubation of the m-Endo LES agar plates also had an effect on the coliform counts, higher numbers of organisms being seen when incubation was at 30 C. The higher counts at reduced temperature would be indicative of the immediate environmental origin of the coliforms isolated as opposed to recent fecal contamination.

Another attempt at coliform isolation involved surface counts from small redwood blocks. This procedure showed large numbers of coliforms (in this case, Enterobacter agglomerans) present on the outer wood surface.

Serial cuts and impressions made through the depth of the wood blocks yielded coliform organisms throughout the wood. Again, higher counts were seen at 30 C than at 35 C, using the selective, differential m-Endo LES medium. All coliform isolates were identified as Enterobacter agglomerans.

Another approach used was a relatively short (15 min) wash period of redwood chips in a Tris buffer solution. By using a shaker and short time span, it was hoped that viable organisms could be released before the phenolic compounds appeared in inhibitory concentrations. In one part of this experiment involving three different incubation temperatures, slightly higher coliform counts were seen at 30 C than at 35 C. These experiments provided some of the highest coliform counts. Also noted was the very slow growth (48 hr to appear) and tiny colony size of most of the coliforms seen on m-Endo LES agar. All isolates were identified as either budding yeasts (which also give a green sheen appearance on m-Endo LES agar) or Enterobacter agglomerans.

In all of these early experiments, Klebsiella species were not isolated. It is believed, however, due to the large numbers seen in water storage tanks (including the laboratory tanks) that Klebsiella species should be present in redwood. Furthermore, the continual reoccurrence and increasing numbers of both Klebsiella and Enterobacter in experimental laboratory tanks would indicate that they are deriving some growth factors from the wooden tanks.

A few hypotheses concerning the absence of Klebsiella isolates in the preceding experiments can be proposed. Possibly, Klebsiella organisms possess a faster die-off rate than do Enterobacter species. Most of the wood samples used in the earlier studies were of indefinite

age, at least 1-2 months old. Also, Klebsiella may be more sensitive to the acidic compounds released when the redwood is examined, hence reducing viable counts obtained. However, there is no direct evidence for these proposals. Finally, Klebsiella is known to be outnumbered by Enterobacter species in natural habitats (2). In the absence of a differential medium, Klebsiella may go undetected.

In order to obtain the freshest redwood samples possible, the sampling trip to northern California was undertaken. The set of extraction experiments performed on these samples was somewhat more successful in obtaining K. pneumoniae, thus demonstrating possible proof that Klebsiella is present in redwood but dies off rapidly as the wood ages.

The use of a better enrichment technique on these fresh redwood samples was also successful in the isolation of K. pneumoniae. Using a modified mFC broth and an elevated temperature of incubation (37 C) greatly enhanced the recovery of Klebsiella from a number of different redwood samples (Table 3-F).

This entire set of experiments dealing with the source of coliform organisms was not completely successful, but did show areas that could be further investigated with better isolation techniques. Both Klebsiella and Enterobacter are present in redwood as part of the indigenous bacterial flora. These organisms survive within the wood structure as the tree is harvested, the wood processed, and are

still viable when the redwood is used in the construction of a water storage tank. With the leaching of growth factors from the wood into the stored water, a suitable nutritive, moist environment results. The coliforms are then able to leave the wood structure and increase in numbers in the water stored in the tank.

Further proof toward the presence of coliform growth factors in redwood will be presented in the next chapter.

TAXONOMY OF COLIFORM ORGANISMS ISOLATED FROM REDWOOD WATER SYSTEM STUDIES

Abstract

During all phases of this redwood water system study, coliform isolates were identified and maintained for further examination. The pertinent biochemical reactions of these isolates were examined following criteria developed by Edwards and Ewing. Some 51 isolates of Klebsiella pneumoniae and 37 isolates of various Enterobacter spp. were examined in this manner. Various compounds contained in redwood, such as carbohydrates and cyclitols, were shown to be utilized as nutrient sources by a high percentage of these coliforms. The response of all K. pneumoniae isolates to the fecal coliform test showed 51% to be positive.

Materials and Methods

All cultures examined were those preliminarily identified as species of the genera Klebsiella and Enterobacter. This identification was based on the typical green sheen colony appearance of coliforms on m-Endo LES medium. Species identification was based on the presence or absence of lysine and ornithine decarboxylase, and arginine dihydrolase. Isolates were stored frozen in glycerol until further studies could be performed. The cultures were grown on Bacto nutrient agar plates following removal from glycerol (24 hr at 35 C). For inoculation in the various taxonomic media, the cultures were grown either in nutrient broth or agar slants (18 $\pm$ 2 hr at 35 C).

All taxonomic tests run on the environmental coliform isolates followed guidelines developed by Edwards and Ewing in their text "Identification of Enterobacteriaceae" (8). The tests included in this study were: indole, methyl red, Voges-Proskauer, Simmons' citrate, H_2S (TSI media), urease, KCN, motility, gelatin, lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, phenylalanine deaminase, utilization of malonate, production of acid and gas from glucose, lactose, and inositol, production of acid from sucrose, mannitol, dulcitol, arabinose, raffinose, rhamnose, and glycerol.

Also included were compounds found in redwood water-soluble extracts, which included some of those recommended by Edwards and

Ewing. These included acid from arabinose, raffinose, rhamnose, sucrose, and glucose, and acid and gas production from inositol. One additional compound, a cyclitol extracted from redwood, was included; the production of acid and gas from pinitol. All carbohydrates were tested at 1% using Bacto phenol red broth base as the indicator system. Pinitol was tested at 0.5% due to the scarcity of the purified compound.

One other factor examined was the membrane Fecal Coliform (mFC) test as developed by Geldreich, Clark, Huff, and Best (11). On this medium, isolates producing blue colonies at 44.5 C are defined as fecal coliforms. Any colonies which are grey, cream, or pink in color are considered non-fecal coliforms.

For this test, all Klebsiella isolates to be examined were grown on Bacto nutrient agar slants for 18 $\pm$ 2 hr at 35 C. One loopful of culture was then resuspended in a small amount of sterile distilled water and streaked on the surface of a mFC agar plate. These plates were incubated at 44.5 C and examined at 24 and 48 hr.

Results

All environmental Klebsielleae (specifically, the genera Klebsiella and Enterobacter) were obtained from samples during all aspects of this investigation. Suspected coliform colonies were streaked on m-Endo LES medium to check for typical green sheen

appearance. The selective m-Endo LES agar detects coliforms due to the fermentation of lactose as the sole carbon source. The presence of ethanol and bile salts tends to exclude non-coliforms. Aldehydes resulting from lactose fermentation react with the basic fuchsin to produce the green sheen appearance. The other tests used to differentiate between Klebsiella and Enterobacter were the ability to synthesize lysine and ornithine decarboxylases and arginine dihydrolase. Motility of the cultures was also examined to verify the results seen with the amino acids.

Table 4-A details the various members of the tribe Klebsielleae that were encountered during this study. Also included are the patterns of amino acid breakdown characteristic of each species and the number of each species examined. One species, Enterobacter agglomerans, while not included in the latest edition of Edwards and Ewing, has recently been described in a paper by Ewing and Fife (10). The taxonomic criteria outlined in that paper have been followed in the present work.

Table 4-B lists the various sources of the environmental coliforms that were examined in the taxonomic tests. As many biochemical tests as possible were performed to make the taxonomy of these environmental isolates comparable to clinical isolates. The results of these tests are found in Tables 4-C (Klebsiella pneumoniae) and 4-D (Enterobacter spp.). In both tables, the percentages

Table 4-A. Patterns of amino acid decarboxylase and dihydrolase activities exhibited by members of the tribe Klebsielleae.[a]

Species	Lysine	Ornithine	Arginine	No. of isolates examined in this study
Klebsiella pneumoniae	+	-	-	51
Enterobacter cloacae	-	+	+	7
Enterobacter aerogenes	+	+	-	9
Enterobacter agglomerans	-	-	-	21

[a]Based on values given by Edwards and Ewing (8) (or by Ewing and Fife (10) in the case of Enterobacter agglomerans).

Table 4-B. Sources of coliform isolates examined in this study.

Origin	Strain
Klebsiella pneumoniae (51 isolates)	
Field Tank SC (Reedsport)	SR 2
Field Tank EC (Eugene)	EC 1
Field Tank EA (Eugene)	EA 2
Field Tank MH (Klamath Falls)	MOY 5
Field Tank SLV (Klamath Falls)	SL 3
Field Tank PW (Klamath Falls)	PC 2, PC 4
Field Tank KRA (Klamath Falls)	K 2, K 3, K 5
Field Tank BW (Klamath Falls)	BW 3
Field Tank WP (Corvallis)	A 1
Lab Tank A (OSU lab)	209, 6, 7, 104
Lab Tank B (OSU lab)	5, 8, 9, 13, 14, 15, 1, 2, 4, 101, C
Lab Tank C (OSU lab)	13, A, B, 9, 11, 12, 14, 102
Redwood sawdust	X 4
Redwood samples from mill at Arcata, California	CT 1, CT 6B, C 1, F, C 2, E44.5 1, E44.5 2, E 37, CT 7, CT 14, CT 11, CT 10, CT 26, CT 43, CT 9
Enterobacter spp. (37 isolates)	
Field Tank SWB (Springfield)	SWB 1, SWB 2, SWB 3
Field Tank SC (Reedsport)	SR 3
Field Tank EB (Eugene)	EB 1, EB 2
Field Tank MH (Klamath Falls)	MOY 2, MOY SL, MOY 4
Field Tank WP (Corvallis)	A 5
Redwood sawdust	RSH 2, RST 2, RSH 5, RSH 1, RSH 3, RST 1, RST 4
Lab Tank B (OSU lab)	B 6, TB 1, TB 2, D, B 1, B 2
Lab Tank C (OSU lab)	15
Wide ring count redwood	WRC 1, WRC 2, WRC 3
Heavy sapwood redwood	HSW 3, HSW 5, HSW 7, HSW 8, HSW 9, HSW 10, HSW 11, HSW 12
Redwood samples from mill at Arcata, California	CT 6C, CT 6A, F 37

Table 4-C. Cultural reactions of Klebsiella pneumoniae isolates compared to data given by Edwards and Ewing (8).[a]

Test or substrate	Environmental (% +)	Clinical (Edwards and Ewing) Sign[b]	Clinical (Edwards and Ewing) % +
Indole	35	-	6.0
Methyl red	14	- or +	13.3
Voges Proskauer	92	+	91.1
Simmons' citrate	100	+	97.7
H_2S (TSI)	18	-	0
Urease	78	+	94.5
KCN	71	+	97.7
Motility	0	-	0
Gelatin	5	-	3.3
Lysine decarboxylase	100	+	97.2
Arginine dihydrolase	0	-	0
Ornithine decarboxylase	0	-	0
Phenylalanine deaminase	0	-	0
Malonate	80	+	92.5
Glucose: Acid	100	+	97.0
Gas	94	+	96.5
Lactose: Acid	100	+	98.2
Gas	100	+	98.0
Sucrose	100	+	98.9
Mannitol	100	+	100.0
Dulcitol	69	- or +	31.5
Inositol: Acid	100	+	97.9
Gas	80	+	91.9
Arabinose	100	+	99.9
Raffinose	94	+	99.7
Rhamnose	83	+	99.3
Pinitol: Acid	92		
Gas	44		
Glycerol	100	+	93.0
mFC response	51		

[a] Of 51 total K. pneumoniae isolates, 36 were run on all tests given above. The remaining 15 were tested in: indole, methyl red, Voges Proskauer, Simmons' citrate, H_2S (TSI), urease, motility, lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, phenylalanine deaminase, malonate, glucose, and mFC response.

[b] From Edwards and Ewing, the sign indicates: +, 90% or more positive in 1 or 2 days; -, 90% or more negative; - or +, majority negative.

Table 4-D. Cultural reactions of environmental Enterobacter spp. isolates compared to standard results.[a]

Test or substrate	E. aerogenes		E. agglomerans		E. cloacae	
	Env.	Std.	Env.	Std.	Env.	Std.
Indole	22	- 0	5	- 19.7	0	- 0.5
Methyl red	22	- 0	76	+ 46.3	0	- 0.3
Voges Proskauer	89	+100.0	81	+ 64.8	100	+ 99.5
Simmons' citrate	100	+ 93.7	90	+ 61.8	100	+ 99.5
H_2S (TSI)	0	- 0	0	- 0	0	- 0
Urease	44	- 2.7	14	- 25.8	71	+ 64.7
KCN	100	+ 98.7	14	+ 40.0	100	+ 98.0
Motility	100	+ 97.3	100	+ 87.5	100	+ 94.5
Gelatin	62	- 0	5	- 3.9	0	- 1.0
Lysine decarboxylase	100	+ 98.7	0	- 0	0	- 0.5
Arginine dihydrolase	0	- 0	0	- 0	100	- 96.5
Ornithine decarboxylase	100	+ 98.7	0	- 0	100	+ 96.0
Phenylalanine deaminase	0	- 0	9	± 27.6	0	- 0
Malonate	89	+ 74.7	90	+ 65.3	100	+ 80.6
Glucose: Acid	100	+100.0	100	+100.0	100	+100.0
Gas	44	+100	62	- 19.1	71	+ 86.0
Lactose: Acid	100	+ 92.3	100	± 44.6	100	+ 93.5
Gas	50	+ 91.0	62	± 24	66	+ 90.0
Sucrose	87	+100	100	+ 71.7	83	+ 96.5
Mannitol	100	+100.0	100	+100.0	100	+100.0
Dulcitol	62	- 4.0	76	- 12.3	17	- 12.9
Inositol: Acid	100	+100.0	95	- 18.3	100	- 21.9
Gas	25	+100.0	0	- 1.4	0	- 5.0
Arabinose	100	+100	96	+ 97.2	100	+ 99.5

(Continued on next page)

Table 4-D. (Continued)

Test or substrate	*E. aerogenes*		*E. agglomerans*		*E. cloacae*	
	Env.	Std.	Env.	Std.	Env.	Std.
Raffinose	75	+ 96.0	90	± 28.5	100	+ 92.0
Pinitol: Acid	87		52		17	
Gas	25		5		5	
Glycerol	100	+100.0	100	± 22.5	66	± 43.3

[a]The standard reactions in the cases of *E. aerogenes* and *E. cloacae* are those of Edwards and Ewing for clinical isolates (8). The reactions in the case of *E. agglomerans* are those of Ewing and Fife for both environmental and clinical isolates (10).

published in Edwards and Ewing (8) (or Ewing and Fife (10) in the case of E. agglomerans) are included for comparison.

A number of compounds found in redwood extractives were examined for possible utilization by the environmental coliform isolates. These compounds were delineated in a paper by A. Anderson, "The Influence of Extractives on Tree Properties" (3). The structures of these compounds are given in Figure 4-A. These compounds are all normally found in the water soluble redwood extract. Those examined in this study included the cyclitols (45% of total water soluble extracts) and carbohydrates (1.5%). Many of the simple sugars were already part of the enteric differentiation scheme of Edwards and Ewing. These include arabinose, glucose, rhamnose, sucrose, and raffinose. The results of these tests for the environmental isolates are included in Tables 4-C and 4-D.

The phenols, which make up 35% of the water soluble extracts, were also examined but problems developed in finding a suitable indicator system. Gallic acid and phloroglucinol were both very unsatisfactory using the phenol red broth base indicator, due to their highly acidic nature. Vanillin, which did mix with the indicator reasonably well, was completely inhibitory to all Klebsiella and Enterobacter isolates at the concentration used (1.0%). There was no growth of any of the cultures after 72 hr at 35 C.

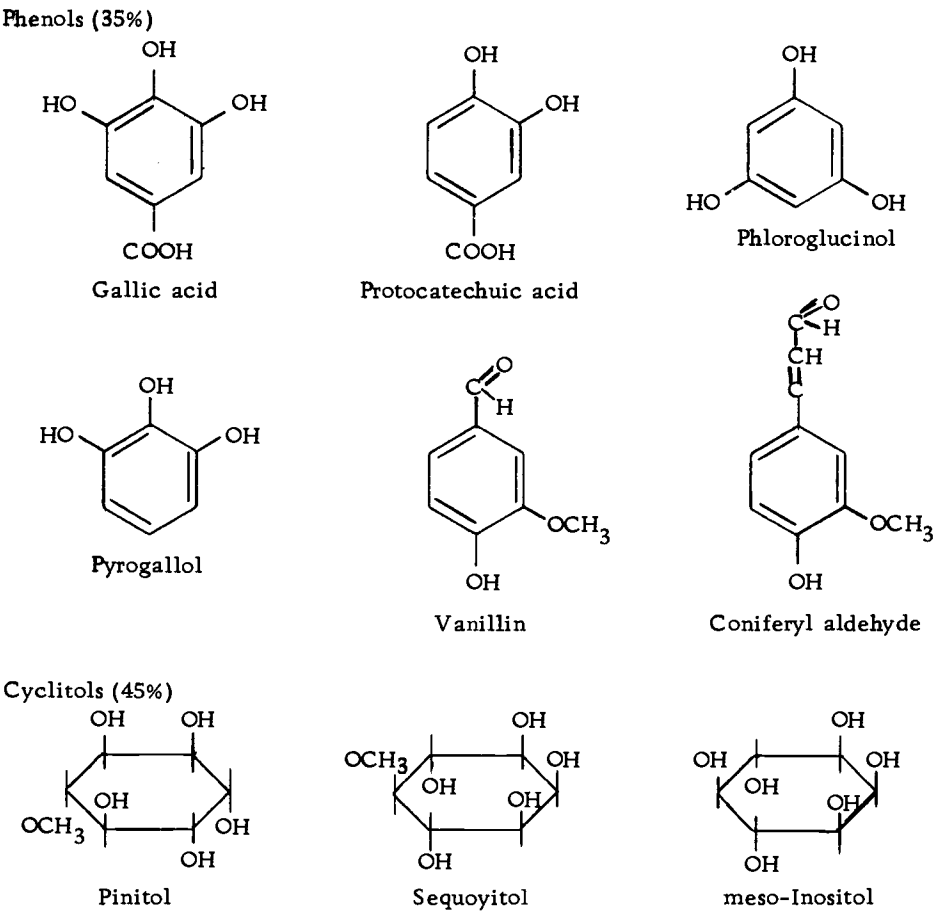
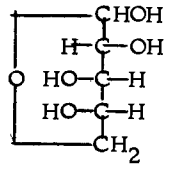
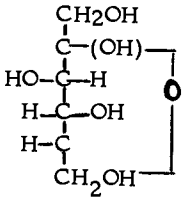


Figure 4-A. Chemical compounds present in water-soluble redwood extracts (percentage of total water-soluble extracts).

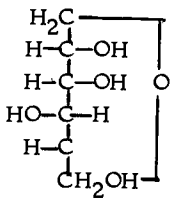
Carbohydrates (1.5%)



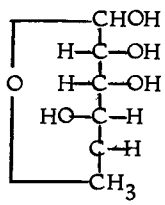
L-arabinose



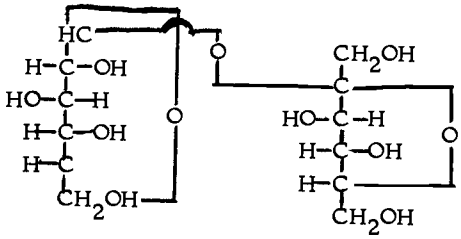
D-fructose



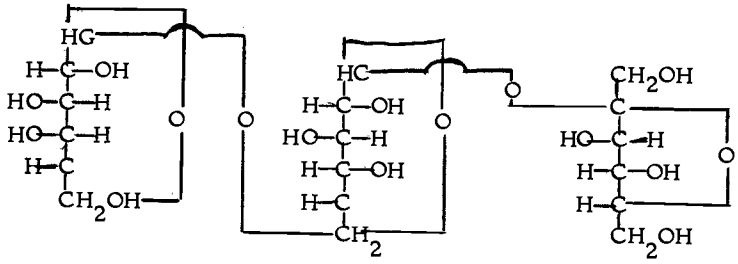
alpha-D-glucose



L-rhamnose



Sucrose



Raffinose

Figure 4-A. (Continued)

The cyclitol compounds present in redwood extracts were also examined. Inositol is a common enteric differential carbon source and was easily obtainable. Pinitol and sequoyitol are rarely purified and both were hard to acquire. A very small sample of pinitol was obtained from Dr. Zavarin (University of California, Berkeley). Unfortunately, no sequoyitol was available. As seen in Table 4-C, 30 of 36 (84%) of the Klebsiella pneumoniae isolates produced acid from pinitol; 44% also produced gas. Using inositol, 100% of these same 36 isolates produced acid; 80% also produced gas.

The cultural reactions of the Klebsiella isolates in the utilization of the carbohydrates are not significantly different from those values published by Edwards and Ewing (8). These environmental isolates utilized sucrose 100%, glucose 100%, arabinose 100%, raffinose 95%, and rhamnose 83%.

In the basic differential tests involving Enterobacteriaceae, the environmental isolates of K. pneumoniae showed three tests with results significantly different from Edwards and Ewing's (8) clinical isolates. These included the production of H_2S on TSI media; clinical isolates do not produce it, while the environmental isolates have a percentage of 18% (9 of 51 cultures examined). Also, 35% of the environmental isolates produced indole from tryptophan, while a value of 6% was given in Edwards and Ewing. Finally, the presence of urease is listed at 94.5% for the clinical isolates, while the isolates in this study showed only 40 of 51 positive, or 79%.

Another aspect of the environmental K. pneumoniae isolates examined was their response to the fecal coliform test on mFC agar medium. Table 4-C includes the results of this test. Twenty-six of the 51 isolates examined (51%) gave a positive response.

Early in this study of redwood water systems, 11 isolates identified as Klebsiella pneumoniae were sent to the Center for Disease Control in Atlanta, Georgia for serotyping. These results are presented in Table 4-E. Of these 11 isolates, two belonged to Type 6, two to Type 51, two to Type 45, and five to a group related to Types 21 and 26. Five of these 11 isolates were subsequently used in this taxonomic study.

The taxonomic characteristics of the various Enterobacter species were only examined closely with regard to the compounds present in redwood extracts. They were, however, run through all the taxonomic tests used for the Klebsiella isolates for completeness. The carbohydrates were fermented by nearly all isolates, and the cyclitols were also utilized by approximately 75% of the cultures. The results are listed in Table 4-D.

Table 4-F includes the results of the taxonomic study of various fungal isolates that were obtained from redwood or tank water. Isolates were also obtained from expressed liquid from redwood blocks. The sources of these isolates are also given in this table.

Table 4-E. Results of serological typing of 11 environmental *K. pneumoniae* isolates.[a]

Strain (OSU code)	Source	Serotype
222	Laboratory Tank B	Type 6
228	Laboratory Tank C	Related to Types 21 and 26
218	Laboratory Tank A	Related to Types 21 and 26
209	Laboratory Tank A	Type 6
103	Laboratory Tank C	Type 51
13	Laboratory Tank C	Related to Types 21 and 26
A	Laboratory Tank C	Related to Types 21 and 26
B	Laboratory Tank C	Type 45
5	Laboratory Tank C	Type 51
2 A	Field Tank (Albany)	Related to Types 21 and 26
A	Field Tank (Albany)	Related to Types 21 and 26

[a]Work performed by the Center for Disease Control located in Atlanta, Georgia.

Table 4-F. Fungal isolates obtained during study of redwood water systems.[a]

Source	Strain Code	Identification
Redwood liquid extract	STM 1	Penicillium sp. "D"
	STM 2	Penicillium sp. "B"
	STM 3	Penicillium sp. "E"
	STM 4	Monilia sp.
	STM 5	Sclerotium sp.
	STM 6	Pesotum sp.
	STM 7	Pesotum sp.
	STM 8	Pesotum sp.
	STM 9	Sclerotium sp.
	STM 10	Pesotum sp.
	STM 11	Penicillium sp. "F"
	STM 12	Pesotum sp.
	STM 13	Penicillium sp. "C"
	STM 14	Penicillium sp. "B"
	STM 15	Aureobasidium pullulans
	STM 16	Penicillium sp. "C"
	STM 17	Cladosporium herbarum
	STM 18	Penicillium sp. "A"
	STM 19	Rhizopus stolonifer
	STM 20	Penicillium sp. "A"
	STM 21	Penicillium sp. "B"
	STM 22	Penicillium sp. "G"
Field tank WP (Corvallis)	WP A	Fusarium oxysporum
	WP B	Mucor sp.
	WP C	Penicillium sp. "H"
Field tank BW (Klamath Falls)	BW 1	Phoma sp.
	BW 2	Fusarium sp.
	BW 3	Mycelia sterilia
Field tank MH (Klamath Falls)	MOY 1	Penicillium sp. "J"
	MOY 2	Fusarium sp.
Field tank EC (Eugene)	EC 1	Penicillium sp. "I"
	EC 2	Aureobasidium pullulans
	EC 3	Penicillium sp. "I"
Field tank EA (Eugene)	EA 1	Penicillium sp. "A"
Field tank EB (Eugene)	EB 2	Phialophora sp.
	EB 3	Mycelia sterilia
	EB 4	Mycelia sterilia

[a]Identification done by H. J. Larsen, Jr., Oregon State Dept. of Botany.

Discussion

In this chapter, coliform isolates taken from all aspects of this investigation of redwood water systems were examined for bacic cultural characteristics. The results proved all isolates to be members of one tribe of the Enterobacteriaceae; the Klebsielleae. Furthermore, all isolates specifically belong to two genera, Klebsiella and Enterobacter.

A thorough taxonomic study was undertaken, conforming to the schema of Edwards and Ewing, "Identification of Enterobacteriaceae" (8). Percent positive fermentation patterns of the environmental isolates were then compared to the results given in the reference tables from the Center for Disease Control (8). In the case of Klebsiella, some major differences were seen, particularly in the tests for production of indole, H_2S, and urease. A higher percentage of environmental Klebsiella isolates produced indole and H_2S than did the clinical isolates.

Increased indole production has been previously documented for Klebsiella isolated in the environment. Indole positive Klebsiella have been found in a wide variety of environmental niches, such as grassland soils of New Zealand (14), associated with decay in living white fir trees (2), on the nodules and surfaces of legume roots (9), on garden vegetables and seeds (4), and on bark from hemlock,

Douglas-fir, and cedar trees (6). In each of these cases, the environmental Klebsiella isolates showed a higher percentage of indole production than seen in clinical isolates.

A number of compounds present in the water soluble extracts of redwood were examined for their utilization by the environmental coliforms. It was seen that, in general, most carbohydrates were utilized by all strains. This had been previously suspected, however, as coliforms in general are known to use these substrates for growth. However, the carbohydrates comprise such a small percentage of the total compounds present in aqueous redwood extracts that they are not of great quantitative importance.

Two larger components of redwood extracts, the phenols and cyclitols, were also tested. The phenols were seen to be quite inhibitory, most probably due to their highly acidic nature. No attempt was made to alter the standard pH indicator system commonly used in these cultural reactions. The cyclitols proved to be quite good substrates for growth. Both the Klebsiella and Enterobacter species utilized them in a high percentage of the cases.

Thus it is seen that these environmental coliforms, found in association with redwood and water stored in redwood systems, can indeed acquire carbon and energy sources from compounds within the wood itself. Specifically, the cyclitols may provide a natural enrichment for the growth of the two genera, Klebsiella and Enterobacter.

Examination of data presented in Edwards and Ewing shows that, besides certain species of Salmonella, Proteus, and Providencia, Klebsiella and Enterobacter are the only coliform organisms that can utilize inositol extensively. Experimental data presented in this chapter show extensive utilization of pinitol also.

Another important point investigated was the response of the environmental Klebsiella isolates to the fecal coliform test. If Klebsiella is able to grow in the water held in redwood tanks, it is expected that any water quality examinations would reveal its presence. A positive coliform or fecal coliform test would render the water unsafe for human consumption under present federal and state standards. It was seen in this study that 51% of these environmental Klebsiella gave a positive response to the mFC test for fecal coliforms under the conditions tested. Hence, they should be considered as indicators of possible fecal contamination.

In the more standard coliform differential tests, the environmental Klebsiella showed a number of differences. However, there are not enough differences seen between the isolates obtained from environmental sources (in this case, redwood and associated water) and those used in Edwards and Ewing's classification (clinical) to make a clear separation. Thus, any Klebsiella isolate found in a drinking water system should be regarded as potentially pathogenic,

regardless of the source. This is especially important as 51% of these isolates do conform to the definition of a fecal coliform.

It has been shown in this chapter that Klebsiella can derive energy for growth from compounds present in redwood and also in water stored in redwood systems. These coliforms come from the wood itself and not from any recent fecal contamination. The presence of the growth promoting compounds for Klebsiellae makes it impossible to assess how recently a hypothetical contamination may have occurred. Contamination could have ultimately been of animal, avian, or insect origin. Klebsiella and Enterobacter have also been isolated from soil and this too must be considered as the origin of Klebsiellae in trees, wood, and wood products.

There is no proof that an environmental isolate of Klebsiella is not as potentially pathogenic as a hospital isolate. Moreover, 51% of these isolates do conform to the fecal coliform definition. There are a few differences in taxonomy between clinical and environmental Klebsiellae, but none that show a clear division. Hence, at this point in time, any Klebsiella isolated from any drinking water sample must be regarded as a potential pathogen and eliminated from the water supply.

DEVELOPMENT OF A 1000 GALLON EXPERIMENTAL REDWOOD WATER SYSTEM

Abstract

A 1000 gallon experimental redwood tank was constructed by National Tank and Pipe Company for use in studies on monitoring a redwood tank system. This tank followed guidelines set up from field and laboratory observations on the parameters necessary for the maintenance of a redwood water system. Routine monitoring included data on chlorine added to the system, residual chlorine leaving the system, water retention time, and microbiological water quality. After 7 months of use at varying retention times, this system has remained coliform-free.

Materials and Methods

For work dealing with an experimental redwood water system, a 1000 gallon tank was constructed by National Tank and Pipe. This tank is shown in Figure 5-A, while the blueprint for its design is shown in Figure 5-B. The tank stands 66 in. high with an interior radius of 36 in. The staves making up the tank are each 2 x 4 in. There is a hinged plywood lid covering the top of the tank.

The incoming water (from Corvallis city lines) is supplied by a pipe entering the center of the tank at the top. This pipe terminates in a spray apparatus which extends approximately 6 in. into the tank interior. The incoming water is directed to all sides of the tank in a spray emanating from approximately 15 small holes in a 1 in. pipe.

The water level within the tank is controlled by a float system which extends from the top of the tank. This float will cause the incoming water to be turned off or on according to a pre-set float level. When the water falls below this level, the float falls, causing the water to be turned on. When the water level rises, causing the float to rise, the water is shut off when the pre-set limit is reached.

Drainage of the tank at specified time intervals was accomplished by a solenoid valve located at the bottom of the tank. An Agastat Motor Timer was used which employed two control dials. One timer controlled the length of time the drain was open. The second

Figure 5-A. One thousand gallon experimental redwood water system located in Withycombe Hall, Oregon State University.

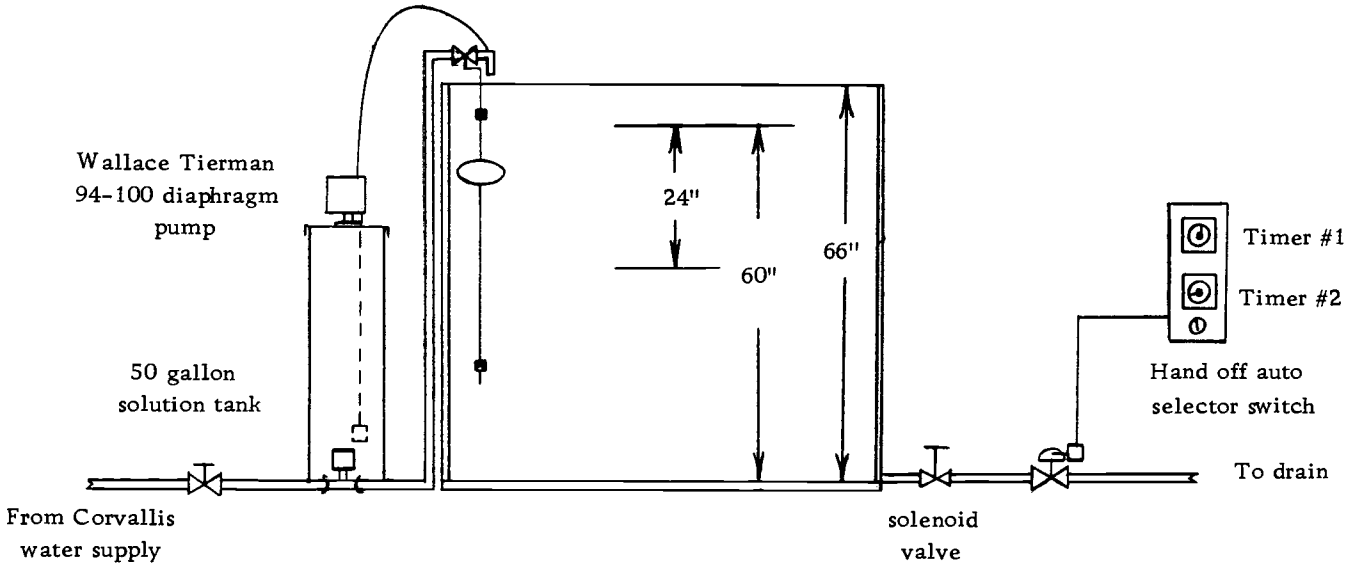


Figure 5-B. Design for 1000 gallon experimental redwood tank.

timer controlled the length of time between drainings of the tank. The shutting off of one timer automatically activated the other. The control panel also included a setting for the continuous manual draining of the tank water. These were used when the tank water needed to be sampled. Generally, a retention cycle had to be interrupted to allow examination of the water entering the tank system.

The total chlorine concentration of the incoming water was regulated using a Wallace-Tiernan solution metering pump. This consisted of a regulatory pump mounted on an 80 gallon tank which held the concentrated chlorine solution. This solution employed laundry bleach (5.25% hypochlorite) as the source of chlorine. It was diluted using tap water as diluent, generally one gallon of bleach to 14 gallons of water (1:15 dilution). A dial located on the pump could be adjusted to allow any chlorine concentration to be added to the incoming water. The chlorine solution was pumped from the storage tank and entered the incoming water supply just prior to its being sprayed into the tank interior. This pump was automatically activated whenever the water entered the tank.

To calculate a desired retention time for the water system, a number of observations were made. The water was observed to leave the tank at the bottom drain at the rate of approximately 12 liters (3.1 gallons)/min. As an example, with a 2-day retention period, the entire 1000 gallon capacity would need to be turned over. This could

be divided up in a number of different ways. Generally, the tank would partially drain 10-12 times/day, for a given period of time. With this 2-day retention, the tank would partially drain 24 times in 48 hr. Each drain period would need to release 1/24th of the tank's capacity, or approximately 42 gallons. At 3.1 gallons/min, this would entail a draining period of approximately 13 min. All other retention schemes were calculated in this manner. Since each draining period would take place for a short time with a full tank, no modifications were necessary to compensate for decreasing flow rate as the tank capacity decreased. This would only have been necessary if the tank had drained to some 50% of its capacity.

The tank itself was assembled in June of 1975 but the chlorine monitoring apparatus was not received until October. The staves had shrunk considerably during this time. This necessitated a period of time in which water was run constantly into the tank in an effort to cause the staves to swell. When this was not completely successful, the staves surrounding the tank were tightened. This stopped most of the leakage, and after a few more days with water held in the tank the leaks had stopped.

After the tank had been completely sealed and leaks effectively eliminated, the sterilization of the tank interior was begun. Following current recommendations of the manufacturer, a soda ash treatment was undertaken to leach phenolic compounds from the wood. This

consisted of adding 5 lb of soda ash to a full tank. This remained for 24 hr. The tank was drained and the wash water replaced by a 200 ppm total chlorine solution which was held for 24 hr. The chlorine was supplied by Hth dry granular chlorine (65% hypochlorite).

For daily monitoring of the tank, samples were taken both from the water leaving the drain at the bottom of the tank and also of the water entering the tank at the top. This was accomplished by interrupting the retention cycle using the control timers. The sample from the bottom of the tank was checked for chlorine residual using the DPD method (16). Also, the pH and optical density were examined. The sample of the incoming water was analyzed for total chlorine concentration using the DPD method.

Periodically, bacterial water quality examinations were performed on water leaving the tank at the bottom drain. A sample was collected in a sterile flask and 100 ml examined for coliforms using membrane filtration and incubation on Bacto m-Endo LES agar at 35 C. A portion of the sample was diluted in sterile distilled water and spread plates made on Bacto nutrient agar at 30 C. Plates were read at both 24 and 48 hr.

The staves within the tank were also periodically examined. Selected staves were scraped using sterile glass slides, then the sample was added to 10 ml of sterile distilled water and agitated. The resulting liquid suspension was streaked (one loopful) onto

m-Endo LES agar for total coliforms. These plates were incubated at 35 C and read at both 24 and 48 hr.

Water samples of approximately 100 ml were periodically removed from the tank and frozen at 0 C. These were saved for later use in a study on nutrient potential of the redwood tank water.

Results

The culmination of experimental research involved studies of a redwood tank of 1000 gallon capacity. An experimental tank of this capacity was designed by National Tank and Pipe, the components delivered to Corvallis, and the tank itself assembled in a large storage area of Withycombe Hall. A photograph of the system and the blueprint of its design are shown in Figures 5-A and 5-B.

The tank was assembled in June of 1975, but due to delays in obtaining a suitable chlorination system, no water was put into the tank until October of that same year. By that time, most of the staves had shrunk to the point where the tank would not hold water. Starting on October 10th, the incoming water was turned on periodically for a few days, in the hope that the staves would swell and seal all leaks. There was some swelling, but not enough to close the tank entirely. On October 14th, the iron bands surrounding the tank were tightened slightly. A few minor leaks stopped after a few days with water held in the tank.

On October 16th, 5 lb of soda ash was added to a full tank of water. This remained in the tank for 24 hr with no circulation. This compound acts to encourage leaching of phenolic and tannin compounds contained in the wood. If these compounds are not adequately removed, problems of taste, odor, and color have been seen to develop in field tank systems.

After 24 hr, the tank was drained and the water observed to be quite reddish in color. Microbiological examination of this water showed no coliforms present, with a total bacterial count of 450 organisms/ml.

On October 18th, the tank was again filled, this time with a solution containing 200 ppm total chlorine concentration. This treatment acts to sterilize the tank interior and prevent development of fungal slime and coliform organisms. When this solution was drained, the water was found to contain a chlorine residual of approximately 175 ppm.

On October 20th, the system was put into operation on a 2-day retention time at 20% tank capacity. This was increased to a 4-day, then 8-day, then 16-day retention, all within 2 weeks of start-up. This was to provide some preliminary data on how much chlorine was needed to maintain a suitable residual at the outflow drain. As can be seen in Table 5-A, a residual of 0.2 ppm was held for most of this period; however, this was brought about only by careful daily

Table 5-A. Data on maintenance of 1000 gallon experimental redwood water system.

Date	Schedule	pH	OD	Cl_2 in (ppm)	Cl_2 out (ppm)	Water quality[b]
October						
20	2-day[a]	7.3	slight color	2.0	0.2	No coliforms, 450/ml total count
21	"	7.4	"	2.0	0.2	
22	4-day	7.2	"	2.0	0.2	
23	"	7.2	"	2.0	0.2	No coliforms, 3000/ml total count
24	"	7.2	"	2.5	0.2	
25	8-day	7.2	"	2.5	0.1	
26	"	7.2	"	3.0	0.1	No coliforms, 2000/ml total count
27	"	7.1	"	3.0	0.1	
28	16-day	7.3	"	3.0	0.1	
29	"	7.3	"	4.0	0.2	
30	"	7.0	"	4.0	0.1	
31	"	6.9	"	4.0	0.1	No coliforms, 2000/ml total count
November						
1	16-day	7.1	"	4.6	0.1	
2	"	7.1	"	4.6	0.1	
3	"	7.3	"	5.0	0.1	No coliforms (water or staves) 3000/ml total count
4	8-day	7.3	"	5.0	0.1	
5	"	7.2	.030	5.0	0.2	
6	"	7.4	.035	5.0	0.2	
7	"	7.4	.035	5.0	0.2	No coliforms, 2000/ml total count
8	"	7.4	.035	5.0	0.2	
9	"	7.3	.040	5.0	0.2	
10	"	7.3	.025	5.0	0.2	No coliforms, 2000/ml total count
11	"	7.0	.040	5.0	0.2	
12	"	6.9	.035	5.0	0.1	
13	"	7.1	.020	5.0	0.2	
14	"	6.9	.020	5.0	0.1	
17	Added 5 lb of soda ash to a full tank (stagnant)					
18		10.7	.010			
19		10.5	.050			
20		10.4	.120			
21		10.4	.160			
24		10.4	.170			
25	Tank again set on 2-day retention after 7 days of soda ash treatment					
26	2-day	7.2	.005	5.0	0.3	
29	"	7.4	.010	5.0	0.2	
December						
1	2-day	7.7	.010	2.0	0.3	
2	"	7.2	.020	2.0	0.3	
3	"	6.6	.010	2.0	0.6	
4	Leak at drain valve repaired					
5	2-day	7.0	.010	1.6	0.2	

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Table 5-A (Continued)

Date	Schedule	pH	OD	Cl_2 in (ppm)	Cl_2 out (ppm)	Water quality
December						
6	2-day	6.6	.005	1.5	0.2	
7	"	6.9	.005	1.5	0.2	No coliforms
8	"	6.9	.005	1.5	0.1	
9	4-day	7.1	.010	1.5	0.1	
10	"	6.6	.010	4.0	0.1	No coliforms, 200/ml total count
11	"	7.1	.015	5.0	0.2	
12	"	7.1	.015	5.0	0.2	
13	"	7.2	.010	5.0	0.2	
14	"	7.1	.020	6.0	0.1	
15	"	7.1	.030	6.0	0.1	
16	"	7.1	.020	6.0	0.1	No coliforms (water or staves) 250/ml total count
17	"	7.1	.030	6.0	0.1	
18	8-day	7.4	.015	12.0	0.3	
19	"	7.3	.020	12.0	0.2	No coliforms, 80/ml total count
20	"	7.8	.015	12.0	0.2	
24	"	7.2	.015	7.0	0.2	No coliforms, 2000/ml total count
25	"	7.1	.015	6.0	0.1	
31	"	7.3	.040	20.0	0.1	
January						
2	8-day	7.1	.010	20.0	0.2	
6	"	6.6	.010	20.0	0.2	No coliforms, 180/ml total count
7	"	7.0	.010	30.0	0.2	
8	"	7.1	.020	30.0	0.2	
9	"	7.0	.010	30.0	0.2	
10	"	7.1	.010	30.0	0.2	No coliforms, 70/ml total count
11	"	7.1	.010	30.0	0.2	
12	"	7.1	.005	20.0	2.0	
13	"	7.0	.005	18.0	1.5	
14	"	7.1	.005	20.0	0.4	
15	"	6.4	.000	25.0	0.6	No coliforms, 10/ml total count
16	"	7.2	.010	15.0	0.1	
20	"	7.3	.010	15.0	0.1	No coliforms, 230/ml total count
21	12-day	7.3	.010	10.0	0.1	
22	"	7.0	.010	8.0	0.2	
23	"	7.0	.020	8.0	0.1	
24	"	6.9	.010	20.0	0.1	No coliforms (water or staves) 20/ml total count
25	"	6.9	.010	20.0	0.1	
26	"	7.0	.015	14.0	0.1	
27	"	6.9	.020	15.0	0.1	
28	"	7.1	.010	15.0	0.2	No coliforms, 10/ml total count
29	"	7.0	.010	30.0	0.2	
30	"	7.0	.010	30.0	0.2	
31	"	6.9	.010	30.0	0.1	

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Table 5-A (Continued)

Date	Schedule	pH	OD	Cl_2 in (ppm)	Cl_2 out (ppm)	Water quality
February						
1	12-day	7.0	.010	30.0	0.1	
2	"	7.0	.005	30.0	0.1	
3	"	7.0	.005	30.0	0.1	
4	"	7.0	.010	30.0	0.1	
5	"	7.0	.010	30.0	0.1	
6	"	7.0	.010	30.0	0.1	
8	Tank filled to capacity[c]					
9	1-day	7.0	.000	20.0	10.0	
10	"	7.0	.000	20.0	5.0	
11	"	7.0	.000	20.0	5.0	No coliforms, 0/ml total count
12	"	7.3	.000	15.0	5.0	
14	"	7.2	.005	15.0	4.0	
15	"	7.1	.005	10.0	4.0	
16	"	7.1	.000	10.0	3.5	No coliforms, 10/ml total count
17	"	7.1	.000	8.0	5.0	
18	"	7.1	.000	8.0	4.0	
19	"	7.0	.005	8.0	4.0	
20	"	7.1	.000	8.0	4.0	
21	"	7.0	.000	6.0	4.0	
22	"	7.0	.000	4.0	3.0	
23	"	7.1	.000	4.0	3.0	
24	"	7.1	.000	3.5	3.0	No coliforms, 0/ml total count
25	"	7.1	.000	3.5	2.5	
26	"	7.0	.000	2.0	1.5	
27	"	7.1	.000	2.0	1.0	
28	"	7.1	.000	2.0	1.0	
29	"	7.0	.000	2.0	1.0	
March						
1	1-day	7.2	.005	4.0	1.5	
2	"	7.1	.000	3.5	2.0	No coliforms (water or staves) 1000/ml total count
3	"	7.0	.000	3.0	1.5	
4	"	7.0	.005	3.0	2.0	
5	"	6.9	.000	2.5	1.0	
6	"	7.0	.000	2.0	1.0	
7	"	7.1	.000	2.0	0.6	
8	"	7.1	.000	2.0	0.4	
9	"	7.1	.000	2.0	0.4	No coliforms, >1000/ml total count
10	"	7.2	.005	2.0	0.4	
11	"	7.1	.005	2.0	0.3	
12	"	7.0	.005	2.0	0.3	
13	"	7.0	.000	2.0	0.3	
14	"	7.1	.000	2.0	0.3	
15	"	7.2	.000	2.0	0.3	
16	"	7.1	.000	2.0	0.2	
17	"	7.1	.005	2.0	0.3	No coliforms, >1000/ml total count

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Table 5-A (Continued)

Date	Schedule	pH	OD	Cl_2 in (ppm)	Cl_2 out (ppm)	Water quality
March						
18	1-day	7.1	.000	2.0	0.3	
19	"	7.0	.000	2.0	0.3	
22	"	7.0	.000	1.8	0.2	
24	"	7.0	.000	1.5	0.2	
26	"	7.0	.000	1.5	0.2	
28	"	7.0	.005	1.5	0.2	
29	"	7.0	.005	1,8	0.2	
30	"	7.1	.000	1.5	0.2	
31	"	7.2	.000	2.0	0.2	
April						
1	1-day	7.1	.000	2.0	0.3	
2	"	7.0	.005	1.8	0.2	No coliforms, > 1000/ml total count
3	"	7.0	.000	2.0	0.2	
4	"	7.0	.000	1.8	0.2	
5	"	7.1	.000	2.0	0.2	
6	2-day	7.0	.005	3.0	0.3	
7	"	7.0	.000	3.0	0.4	
8	"	7.2	.000	3.0	0.3	
9	"	7.3	.005	3.0	0.4	No coliforms, > 2000/ml total count
10	4-day	7.2	.000	3.5	1.0	
11	"	7.2	.000	3.5	1.0	
12	"	7.1	.000	4.0	1.0	
13	"	7.0	.000	4.0	0.6	
14	"	6.9	.000	3.0	0.6	
15	"	7.2	.000	3.5	0.3	
16	"	7.4	.000	3.0	0.2	
17	"	7.2	.000	3.0	0.2	
18	"	7.1	.000	2.5	0.1	
19	"	7.0	.000	2.5	0.1	No coliforms (water or staves) > 2000/ml total count
20	"	7.1	.000	2.5	0.1	
21	"	7.0	.010	4.0	0.2	
22	"	7.3	.000	4.0	0.2	
23	"	7.0	.000	4.0	0.1	
24	"	6.9	.000	5.0	0.3	
25	"	7.0	.000	5.0	2.0	
26	"	7.2	.000	5.0	2.0	
27	"	7.1	.005	5.0	1.5	
28	"	7.0	.005	5.0	1.5	
29	"	7.0	.005	5.0	1.3	
30	"	7.0	.000	5.0	0.5	

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Table 5-A (Continued)

Date	Schedule	pH	OD	Cl_2 in (ppm)	Cl_2 out (ppm)	Water quality
May						
1	4-day	7.1	.005	5.0	0.5	
2	"	7.1	.000	5.0	0.5	
3	"	7.0	.000	5.0	0.5	
4	"	7.1	.000	5.0	0.5	
5	"	7.0	.000	5.0	0.5	
6	"	7.0	.000	5.0	0.5	
7	"	7.1	.000	5.5	0.5	No coliforms, 1/ml total count
8	"	7.0	.000	5.5	0.5	
9	"	7.0	.000	5.5	0.5	
10	"	7.0	.000	5.5	0.4	
11	"	7.0	.005	5.0	0.3	
12	"	7.0	.005	5.0	0.3	
13	"	7.1	.005	5.0	0.1	
14	"	7.0	.000	5.0	0.2	

[a]Tank was put into operation at approximately 20% of 1000 gallon capacity.

[b]Coliform counts per 100 ml samples filtered (m-Endo LES at 35 C). Total counts per ml were on nutrient agar at 30 C. Coliform counts from staves were on m-Endo LES agar.

[c]Tank was operating at approximately 90% of 1000 gallon capacity.

examination of the chlorine content of the tank water and incoming water supply. The maintenance of a desirable 0.2 ppm residual at the 2- and 4-day retention periods in these initial experiments was accomplished with 2.0 ppm total chlorine concentration in the receiving water.

One immediate problem was the need to anticipate the change in incoming total chlorine necessitated by increasing the retention time. This can be seen on October 28th and following days, when the tank was put on a 16-day retention time. The total chlorine concentration of 4.0 to 5.0 ppm was barely sufficient to maintain a residual of 0.1-0.2 ppm. Furthermore, with extended retention times like 16 days, it often takes several days for the water system to equilibrate to new chlorine amendments. This was made obvious during the 8-day retention period used during December 18th to January 20th. The adjustment of the incoming chlorine residual to 30.0 ppm on January 7th was not manifested in the drain water until January 11th when the residual increased 10-fold dramatically (0.2-2.0 ppm). Such a lag in chlorine equilibration could have led to errors (underestimates) with interpretation of the necessary chlorine concentration required to maintain 0.2 ppm chlorine residual during the early study period (October and November).

Water quality examinations during the preliminary work in late October showed no coliforms present either in the water or on stave

surfaces. Total bacterial counts were approximately 2000 organisms/ml.

On November 4th, the system was put back on an 8-day retention time for 10 days. This allowed some time for study of one specific retention scheme. The system appeared to stabilize with an incoming total chlorine concentration of 5.0 ppm and residual concentrations at the drain of approximately 0.2 ppm. However, it appeared that this residual was dissipating (0.1 ppm near the end) when this sequence was terminated. During this specific interval the 8-day retention was accomplished by draining the tank water for 7.5 min at each of three periods per day.

During the early weeks of operation, a slight reddish color and "rain-barrel" odor had been observed in the tank water. This problem did not disappear, and optical density readings (compared to tap water) averaged about 0.035 (550 nm). A second soda ash treatment was deemed necessary.

Five pounds of soda ash were added to a full tank and left for 7 days. There was no circulation of the water. Readings of the pH and optical density of the drainwater were monitored. The pH stabilized at 10.4, while the optical density readings increased from 0.010 to 0.170 by the end of the 7-day period. This represented a tremendous accumulation of leached matter (some 10^6 total optical density units) since the tank was near capacity of 1000 gallons.

On November 25th, the tank was again put into operation on a 2-day retention time. This was slowly increased to a 4-day, then 8-day, then 12-day retention over the next 3 months. No major problems occurred, and the water continued coliform-free as did the interior staves of the tank. Again, observations showed that very careful daily monitoring was quite necessary to the successful management of the tank. Decreasing chlorine residual at the tank drain (due to longer retention periods) had to be compensated for by an increase in the incoming total chlorine concentration. The incoming chlorine concentrations were higher than those needed during the initial study period in October and November, even though the timing of the retention periods was identical. A 4-day retention period necessitated at least 6.0 ppm incoming chlorine to maintain a 0.2 ppm residual at the drain.

The complications inherent in the lag for chlorine equilibration resulted in the variations in adjusting the incoming chlorine concentrations for the 8-day retention period in December and January. It was estimated that 10 to 15 ppm chlorine was necessary to maintain a satisfactory residual with an 8-day retention schedule. For the extended 12-day retention period it was necessary to supply 30.0 ppm chlorine in the receiving water.

On February 7th, the tank was switched to approximately 90% of its 1000 gallon capacity. A 1-day retention period was put into effect

at this time. This was established with 14 drain cycle/day, each draining for 10 min or 7.1% of the tank capacity.

At the time of this change from 20 to 90% tank capacity, an increase in the incoming chlorine concentration was necessitated in order to maintain the desired 0.2 ppm residual at the tank drain. This increase was thought to be due to a number of different factors; possibly because of the increase of drain periods/day (from 3 to 14), the large increase in wood surface area with its high chlorine demand, and also the decrease in percentage of the chlorinated water column contacting the wood itself.

It was not until early March that a suitable equilibrium was reached with the tank operating at the 90% capacity level. For example, on March 14th an incoming total chlorine concentration of 2.0 ppm gave a drain residual of 0.3 ppm.

In April of 1976, the tank schedule was increased to a 2-day retention time and then to a 4-day scheme on April 10th. The tank is still operating on the 4-day retention as of May 14, 1976.

During operation of the tank at full capacity, there were very few problems. Maintaining an adequate residual entailed daily monitoring, with various adjustments made on the incoming total chlorine concentration. This was especially necessary when a dramatic increase in the tank capacity occurred, as the change from 20 to 90% on February 7th.

The water quality examinations have been negative for coliforms at all points. However, total counts on nutrient agar have varied considerably. This is evidently due to bacterial "blooms" within the tank that are set off whenever there may be a drop in chlorine residual. Some budding yeasts have been observed as well as common bacterial types. In all cases where growth occurred on nutrient agar plates, random colonies were picked and streaked onto m-Endo LES agar. This was to insure that no coliforms were growing on the non-selective nutrient agar medium. No coliforms were observed from nutrient agar plates at any point in this study.

In general, the switch in tank capacity from 20 to 90% caused a large increase in the average total bacterial counts seen on nutrient agar. This was due to the great increase in wood surface exposed to the aquatic environment when the capacity was altered.

As early as February of 1965, very sparse patches of fungal slime were observed on some staves of the tank interior. These were quite thin and faint, just barely visible when examined using a flashlight to survey the tank interior. Samples were scraped and suspended in sterile distilled water, then plated on both m-Endo LES and nutrient agars. No coliforms were present.

This slime accumulation increased slowly and in late April was found in larger patches on approximately 10 of the staves. However, no coliform organisms have been found in the slime to date.

Microscopic examination of slime samples has revealed a fungal matrix with some bacteria embedded within. These bacteria have been non-coliform types.

As of May 14th, when this part of the 1000 gallon tank study was completed, the system was operating on a 4-day retention. The incoming chlorine was 5.5 ppm and the drain residual 0.3-0.4 ppm. The slime evident on some staves is not considered to be problem and will continue to be observed as time passes. The system is totally coliform-free and the water is potable.

Discussion

The construction and maintenance of an experimental redwood water system was the final area of investigation in this study. The purpose of these experiments was to demonstrate that a redwood system could be properly designed and successfully store potable water. By carefully observing the day-to-day problems associated with providing potable water, it was hoped that certain guidelines could be developed that would apply to all redwood systems.

Phenolic and tannin compounds that leach from the wood as water is held in the tank can cause user-associated problems of taste, odor, and coloration. It was seen that the single 24-hr treatment with soda ash was not adequate to eliminate these compounds. This treatment should last at least one full week, possibly two, with

periodic amendments to the original soda ash solution. This should serve to leach out as much of these compounds as possible.

Another problem is sterilization of the tank interior to prevent slime and coliform organism development. The 24-hr treatment with a solution of 200 ppm total chlorine seemed adequate during the first part of the study, but the slow development of slime since February has shown this to be incorrect. It may be necessary to surface sterilize with 200 ppm total chlorine every 3 to 6 months during the first 12-24 month history of new tanks to prevent this slime accumulation.

One observation of a field system bears out this idea. The redwood tank system at Whispering Pines Lodge near Corvallis (reviewed in Chapter One) was carefully maintained since its construction, with daily monitoring of the chlorine residual reaching users on line. The manufacturers' recommended pretreatment of 200 ppm total chlorine for 24 hr was followed. However, slow development of fungal slime necessitated further chlorine treatment, in the form of interior washes using a 200 ppm total chlorine solution. These washes were performed after approximately 6 and 12 months of tank use, when slime development necessitated them.

Therefore, even with adequate chlorination of the tank water at all times, further interior sterilization of the tank may be necessary during the first few years of a redwood tank's history. This will

compensate for the slow leaching of nutrients from the wood interior that can allow development of fungal slime and possible coliform organisms.

Regular monitoring of a tank system appears necessary to maintain an adequate chlorine residual to all users on line. The incoming total chlorine concentration must be monitored as well as the residual leaving the system. Retention time of the system must be observed, and all factors considered to provide a residual of 0.2-0.3 ppm.

It may not be possible to extrapolate concentrations of chlorine based on the 1000 gallon system to much larger 100,000 gallon systems. However, the levels of chlorine observed in the experimental system are thought to be close to expectations since the field sampling of tank WP (25,000 gallons) indicated that some 1.5 ppm total chlorine in the incoming water was sufficient to achieve the desired residual at the tap.

The retention time of a field system will vary, especially due to seasonality and increase in user households. The chlorine residual reaching users on line must be checked carefully when such changes do occur, and any decreases below a safe level must be compensated for by an increase in incoming total chlorine.

In conclusion, it has been seen that an experimental 1000 gallon redwood water system can be adequately maintained. The water from

this system has been potable and coliform-free for a total of nearly 7 months. This is a significant improvement when compared to the vast majority of field systems now in use. What is needed to run such a system safely is careful monitoring with the goal of maintaining a chlorine residual of at least 0.2 ppm at all points in the system, regardless of retention time.

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