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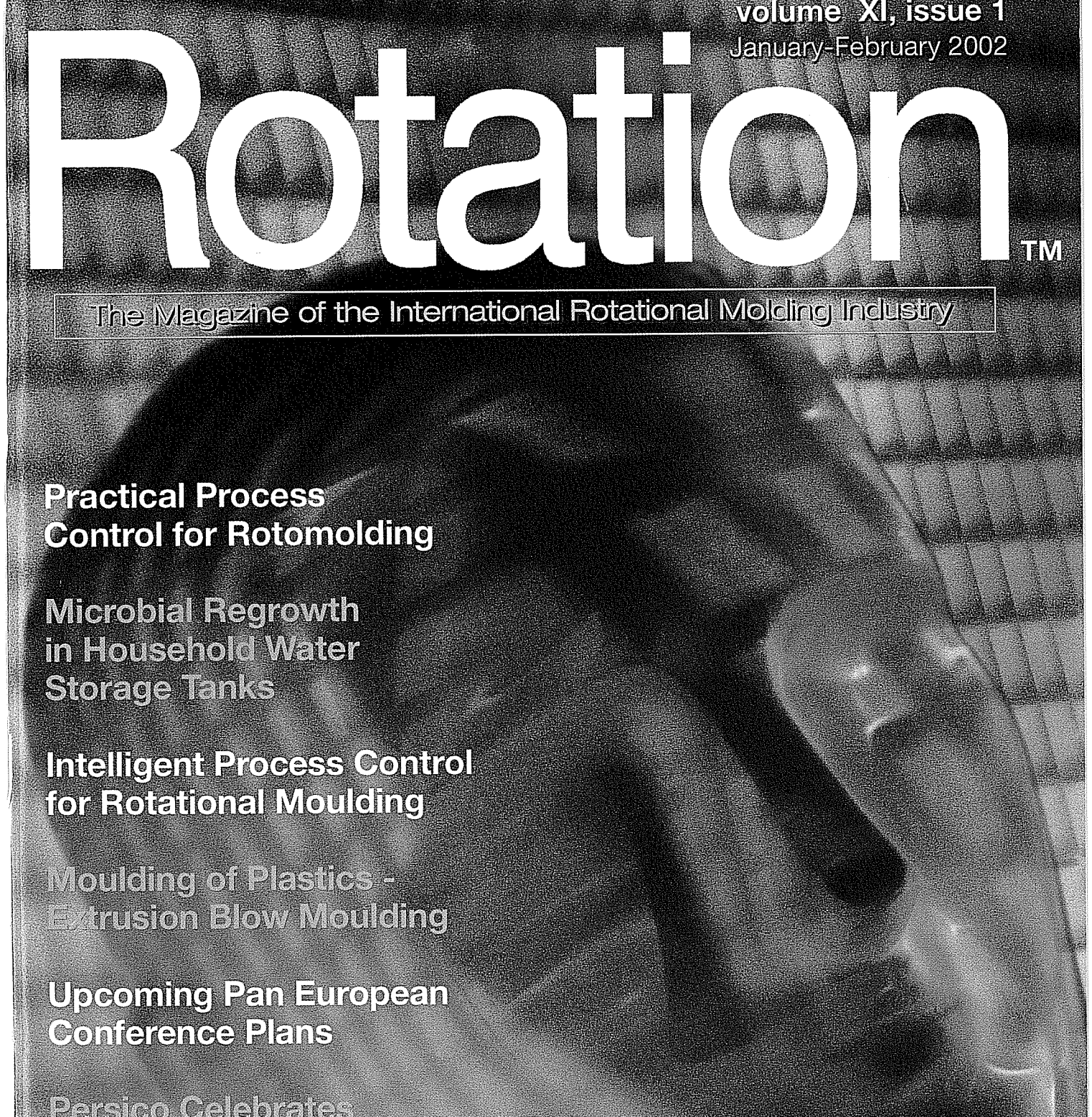


Practical Process Control for Rotomolding

Microbial Regrowth in Household Water Storage Tanks

Intelligent Process Control for Rotational Moulding

Moulding of Plastics - Extrusion Blow Moulding

Upcoming Pan European Conference Plans

Persico Celebrates

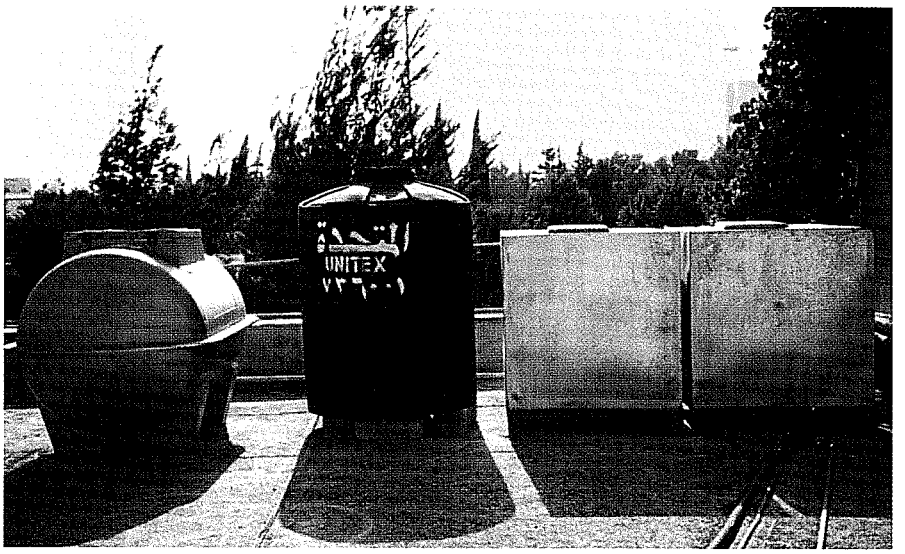
Microbial Regrowth in Household Water Storage Tanks

by Lilian Evison, University of Newcastle upon Tyne and Nawal Sunna, Water Authority of Jordan, Amman, Jordan

Microbial regrowth in water distribution systems and its effect on water quality are emerging concerns within the water supply community. A growing body of evidence suggests that microbial regrowth could cause a public health hazard by encouraging the growth of pathogens or opportunistic pathogens (Rusin et al, 1997; Power, 1995; LeChevallier & McFeters, 1985), interfering with coliform detection and invalidating existing water quality monitoring programs (Camper, 1995; Burlingame et al, 1984), and playing a part in transferring antibiotic resistance factors to pathogenic bacteria (Gaur et al, 1992; Grabow et al, 1975; Grabow et al, 1974).

In countries with unreliable water supplies, such as Jordan, the problem of microbial regrowth is exacerbated by storing the distributed water for periods as long as four to seven days, especially in the summer season. The most commonly used household tanks in Jordan are made of cast iron, polyethylene, or fiberglass. Such factors as long retention times, presence of low or zero chlorine residual, high temperatures, and presence of sediments only increase the likelihood of microbial growth in these household tanks (Schoenen, 1990; Schoenen & Scholer, 1985; LeChevallier et al, 1981; Schoenen & Dott, 1977; Grabow et al, 1975).

The objective of this study was to investigate the extent and characteristics of microbial regrowth in the different types of household tanks as well as the parameters influencing this regrowth.



The four household storage tanks used in the study were made of (from left) fiberglass, polyethylene, and cast iron.

Materials and Methods Described

Tanks of various materials were studied. Four tanks (two cast-iron, one polyethylene, and one fiberglass), each 1 m3 in size, were appropriately installed in series on the roof of the laboratory building, Ministry of Water and Irrigation, Amman, Jordan. Light penetration was higher in the fiberglass tank than in the polyethylene tank; no light penetrated the cast-iron tanks. The influent water to the tanks was surface water that had been conventionally treated (i.e., oxidation, coagulation and flocculation, sedimentation, filtration, and chlorine disinfection).

A water sampling tap was installed on the effluent water pipe of each household water storage tank. Keys with valves were installed on the influent pipeline to control time and flow of the water. An electric pump was used to draw the water from the tanks when needed and to mimic ordinary water use; water was pumped from the tanks for four hours before the tanks were refilled with water each week during the study period. Water drained from the tanks was pumped to the main building water tanks intended for daily use in the laboratory.

Before the start of the experiment, the household water tanks were thoroughly disinfected with hypochlorite disinfectant (70%). After a day of storage, water samples from the four household storage tanks were analyzed for residual chlorine and heterotrophic plate counts (HPCs) to ensure that the tanks were disinfected to the same level. Disinfection was stopped after the plate count was < 100 cfu/mL and was comparable to the nearest 20 cfu/mL in the four household tanks.

The study period included change of seasons. The household tanks were drained and refilled weekly for a period of ten months (September 1996-July 1997). On a bimonthly basis, samples were collected from the tank influent water and from each household water storage tank after the same water had been stored for four and

seven days. Chemical and bacteriological samples were collected as described in Standard Methods (1995).

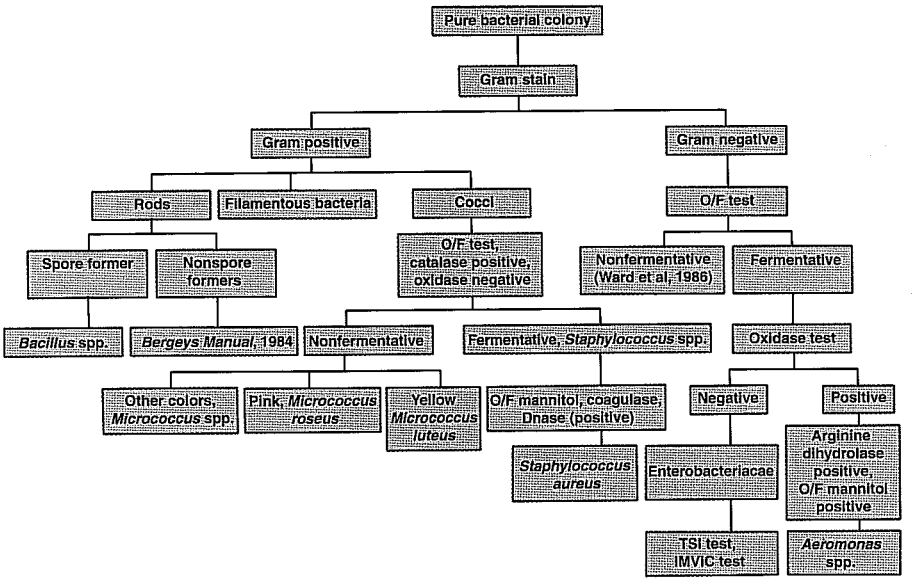
At the end of the experiment, the household tanks were drained by opening the drain tap, and sediment samples for microbial tests were collected by a sampler that consisted of a handle and syringe to withdraw the sediments. A clean new brush was used to scrape the sediment at the bottom surface; sediment was then homogenized in the remaining water below the drain opening (4-10 cm above the bottom surface). Samples were collected from the drain opening for analysis of dry weight, total organic carbon (TOC), chlorophyll A concentrations, and microscopic organisms. The volume of the sediment water in each tank was measured to calculate the rate of sediment accumulation as grams of dry weight/cubic metre of household tank bottom surface.

Water samples were tested for chlorine residual using the DPD colorimetric method and a photometer. Temperature was determined using a calibrated thermometer. Turbidity and pH were determined using a turbidimeter and a pH meter. TOC concentration was determined by persulfate-ultraviolet oxidation method, and a TOC analyzer.

Trihalomethane (THM) concentrations in the distributed water and in the household tank water after four days of storage were measured three times during the study period and were determined using a gas chromatograph. Chlorophyll a concentrations in the sediment samples were determined by the spectrophotometric method using a spectrophotometer. All physicochemical tests were measured as described in Standard Methods (1995).

Total and fecal coliforms were enumerated by the total coliform membrane filtration procedure (Standard Methods, 1995) using m-Endo and m-Fc agar media. Heterotrophic bacteria were enumerated using R2A agar and the spread plate technique. Plates were incubated at 28 °C. Colonies were counted after seven days of incubation using a Quebec colony counter. The Pseudomonas aeruginosa test was performed using the most probable number method as described in Standard Methods (1995).

Microorganism identification was conducted using a flow diagram, which is based on Bergeys Manual (1984), Ward et al (1986), and LeChevallier et al (1980). Microscopic analysis for sediment samples was conducted using a sedimentation technique. Water samples (100 mL) were left to settle for 7 hours; then 1 mL was drawn by pipette from the bottom of each sample.



O/F—oxidation/fermentation; TSI—triple sugar iron (agar); IMViC—I—indole test, M—methyl red test, V—Vosges Proskauer test, C—citrate utilization

Fig. 1. Flow diagram of the identification procedure used to identify R2A plate count bacteria.

Results spotlight factors leading to water degradation

Storage time affected water quality. Results showed that the water underwent significant changes in its physicochemical and bacteriological characteristics after being stored for four and seven days.

Increase in HPCs was accompanied by loss of chlorine residual and an increase in pH. Turbidity and TOC decreased after four days of storage and then increased after seven days' storage. THM concentrations increased during the four-day storage period and reached a maximum concentration of 162 μg/L. Although the THM concentration in the influent water complied with Jordanian standards (Jordanian Drinking Water Standard, 1997) and did not exceed the limit of 150 μg/L, nevertheless the water in the household tank after the storage period violated the standards. No coliforms or P. aeruginosa were detected in the distributed or the household tank water samples during the whole study period (September 1996-July 1997).

Seasonal variation was also an influence. Seasonal factors significantly affected microbial regrowth in the household tanks, with HPCs showing a clear decrease in the winter season (October-April). However, results also showed that the difference in HPCs between four and seven days' storage time was larger at the beginning of the experiment than after the tanks had been in use for 10 months. The bacterial plate count

seems to plateau in the household storage tanks, where the log plate counts at four and seven days' storage time became similar and did not exceed log 5.8 cfu/mL for the last three months.

Turbidity demonstrated a seasonal variation in the household tanks as well. Turbidity increased during the summer season after water had been stored for four and seven days (Figure 2). On the other hand, the turbidity of the influent water showed higher levels during the winter season. The pH of the stored water did not show any seasonal variation (Figure 3).

Characteristics of bacteria found in household water storage tanks. Sixty-nine isolates were identified from the R2A agar plates of the household storage tanks and from the distribution system water samples. The gram-positive group constituted the largest percentage of the bacterial isolates of the household tank water with dominance of Actinomycetes species. More species *(e.g., Aeromonas, Pseudomonas vesicularis, Pseudomonas/Alcaligenes, and Moraxella spp.)* were identified in the stored water than in the influent water samples (Tables 1 and 2).

Results show some influence of tank material on water quality. For all household tank waters, results indicated a significant difference (>0.01) between HPCs at no storage time and those at four and seven days' storage but no significant differences (<0.01) between HPCs at four days of storage and those at seven days. There were no significant differences in the physicochemical parameters, residual chlorine, pH, turbidity, and temperature among the different household tank waters.

Cluster analysis showed a higher similarity in the microbial taxa in the two cast-iron tanks compared with the polyethylene and fiberglass tanks. *Bacillus* spp. and *Moraxella* spp. were found in higher percentages in the cast-iron tanks than in the fiberglass and polyethylene tanks. On the other hand, *Arthrobacter* spp., *Pseudomonas/Alcaligenes*, and *Aeromonas* were found in higher percentages in the fiberglass and polyethylene tanks (Table 1).

Sediment characteristics varied by tank material. The rate of accumulation in the tanks was 30-100 mg dry weight/m3 of water. The sediments of the fiberglass and polyethylene tanks showed higher concentrations of total bacteria, TOC, and plankton than did the sediments of the two cast-iron tanks (Table 3). The highest percentage occurrence of bacterial species identified from sediment samples was for gram-negative nonfermentative bacteria, mainly Pseudomonas spp. (Table 4). Yeast was found in the fiberglass tank.

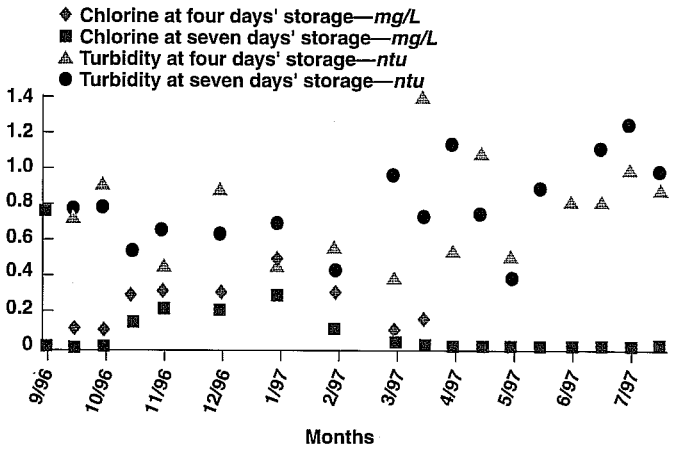


Fig. 2. Variation in the temperature and pH of the water samples at four and seven days' storage time.

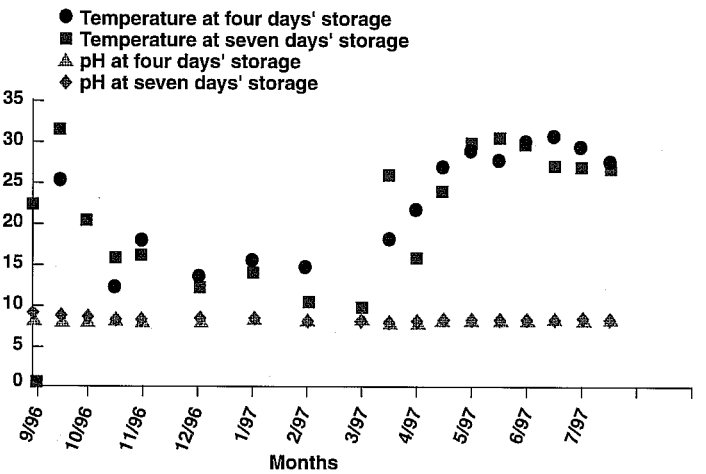


Fig. 3. Variation in the temperature and pH of the water samples at four and seven days' storage time.

Discussion

Temperature and time were influential factors. Study results suggested that microbial growth occurred in the household tanks up to a plateau level (log 5.2-5.8 cfu/mL). The temperature and the time that tanks had been in use were the most important parameters affecting this growth, irrespective of the influent water bacterial numbers. This finding has significant implications in arid countries that adopt an intermittent regime of supplying water. The pattern of bacterial growth in the storage tanks followed the pattern of temperature fluctuation; on the other hand, the tank influent bacterial growth pattern was not affected by seasonal variation. This might be because of the effect of higher chlorine residual and shorter retention time (2-4 hours) in the influent water than in the household tanks, in which chlorine masks the effect of temperature and the short retention time decreases bacterial growth rate in the influent water.

The concentration of TOC in the sediments, mostly attributable to algal and bacterial growth in water (and possibly the sedimentation of particulates in the water), was found to influence the rate of microbial

growth. The time the tank had been in use also reflected accumulated sediments in the tanks. This suggests that improving tank design and preventing light penetration and sediment accumulation may improve the water quality.

Noncontinuous flow may harm water quality. The presence of high HPCs coincided with prevalence of bacteria that could cause taste and odor problems (e.g., *Actinomycetes*) or be considered opportunistic pathogens (e.g., *Moraxella* spp. and *Aeromonas* spp.). The increase in these bacterial species may be attributable to their ability to grow slowly under low-nutrient water conditions. In previous studies, *Moraxella* spp. were also found at dead-end locations with long retention time in distribution systems (Gibbs, 1990), a situation comparable to that found in household storage tanks. Given this finding, actions should be taken to minimize the effect of water storage in household tanks in which a continuous flow cannot be met because of water shortage conditions.

Previous studies have emphasized the importance of the sediment-water interface as a growth-promoting environment that offers greater availability of nutrients and surfaces, thus helping bacteria establish, grow, and multiply (Schreiber & Schoenen, 1994; Allen et al, 1979).

This study found no difference between total bacterial counts in the sediment and water samples. This may be attributable to the size of the household tanks used in this study; their smaller size provided for a better mixing process than can be found in the service reservoirs. Schreiber and Schoenen (1994), however, reported significant differences in bacterial plate numbers between sediments and water samples.

Sediments provide environment for organisms. Microscopic examination showed that the sediments of the household tanks constituted a complete environment in which the nematodes, insect larvae, protozoa,

Bacterial Genera/Species	All Tanks	Cast-iron 1	Cast-iron 2	Fiberglass	Polyethylene
Actinomycetes	17.3	31.6	8.3	24.2	14.7
Aeromonas	9.6		0.3	13.8	19.0
Alcaligene-like (CDC vd-1, CDC vd-2)	0.3			0.9	
Arthrobacter	3.8			6.2	6.7
Bacillus	11.2	23.7	16.5	6.2	6.7
Flavobacterium	1.2		3.8		
Flavobacterium aquatile	0.1	0.1			
Klebsiella rhinoscleromatis	3.6			11.9	
Micrococcus luteus	0.1		0.2		
Micrococcus roseus	0.1	0.1			
Micrococcus spp.	0.1		0.2		
Moraxella	5.9	12.2	15.5		
Moraxella nonliquefaciens	2.7				10.2
Moraxella phenylpyruvica	3.7		7.7		5.1
Pseudomonas diminuta	0.3			0.9	
Pseudomonas vesicularis	3.1	0.2			11.8
Pseudomonas/Alcaligenes	5.6		3.7	13.7	
Pseudomonas spp.	0.1	0.1		0.0	0.1
Staphylococcus spp.	0.1				0.1
Identified isolates	49.1	67.8	56.3	77.9	74.4
Unidentified isolates*	50.8	32.2	43.7	22.1	25.5
Number of isolates		13.0	14.0	19.0	22.0

*Unidentified isolates consisted of 20% gram-negative nonfermentative bacteria for each tank; 1% and 2% gram-negative fermentative rods for cast-iron tanks 1 and 2; and 10%, 8%, and 5% of isolates that failed to grow on subsequent subculturing in cast-iron 2, fiberglass, and polyethylene tanks.

Table 1. Percentage of bacterial genera/species isolated and identified from the four household water tanks during the period (September 1996 - July 1997).

	No Storage	Four Days' Storage—*average (range)*				Seven Days' Storage—*average (range)*			
Parameter	Average *(range)*	Cast-iron 1	Cast-iron 2	Polyethylene	Fiberglass	Cast-iron 1	Cast-iron 2	Polyethylene	Fiberglass
Temperature—*°C*	21.7 (14–27)	20.3 (8–29.8)	20.4 (8–29.8)	20.7 (9–30.0)	21.2 (9–31.5)	21 (8–29.8)	20.4 (9–32.0)	20.7 (9–30.0)	21.4 (10–31.5)
pH	7.7 (7–8.03)	7.9 (7.6–8.2)	7.9 (7.6–8.2)	7.9 (7.6–8.1)	7.9 (7.8–8.1)	8.1 (7.7–8.6)	8.0 (7.7–8.2)	8.0 (7.6–8.2)	8.0 (7.7–8.3)
Turbidity—*ntu*	0.8 (0.2–2.3)	0.7 (0.4–1.1)	0.5 (0.0–2.0)	0.7 (0.4–2.3)	0.7 (0.3–1.6)	0.7 (0.3–1.0)	0.5 (0.0–2.0)	0.7 (0.3–1.4)	0.7 (0.3–1.7)
Cl_2*—*mg/L*	0.6 (0.5–1.0)	0.1 (0.0–0.5)	0.1 (0.0–0.5)	0.1 (0.0–0.5)	0.1 (0.0–0.5)	0.0 (0.0–0.5)	0.1 (0.0–0.5)	0.05 (0.0–0.3)	0.1 (0.0–0.3)
Log HPC†—*cfu/mL*	1.4 (0.9–1.2)	3.0 (0.5–5.7)	3.1 (1.0–5.7)	3.3 (1.1–5.7)	3.2 (1.4–6.7)	3.7 (1.0–6.08)	3.1 (1.0–5.7)	4.3 (1.3–6.8)	4.0 (1.9–6.8)
TOC‡—*mg/L*	1.95 (1.2–2.1)	1.9 (1.0–2.9)	1.6 (1.0–2.1)	2.1 (2.2–2.7)	1.7 (1.1–2.2)	2.7 (2.3–3.2)	1.6 (1.0–2.1)	3.2 (2.4–3.9)	3.3 (2.3–4.3)

*Cl_2—free residual chlorine
†HPC—heterotrophic plate count
‡TOC—total organic carbon

Table 2. Averages and ranges of water quality characteristics of the household water storage tanks during the study period (September 1996 - July 1997).

Parameter	Cast-iron 1	Cast-iron 2	Fiberglass	Polyethylene
Log plate count—*cfu/mL of sediment sample*	5.6	6.89	6.21	6.13
Sediment density—*g/m²*	4.0	2.4	4.0	9.0
Chlorophyll a—*µg/g*	32	85	215	81
TOC*—*mg/g of sediment sample*	30.2	41.5	73.3	46.0
Nematode—*unit/100 mL of sediment sample*	10	25	10	15
Cysts—*unit/100 mL*	15	5	15	15
Plankton—*unit/100 mL of sediment sample*	60	65	90	80
Protozoa—*unit/100 mL of sediment sample*	20	15	TNC†	15

*TOC—total organic carbon
†TNC—too numerous to count

Table 3. Sediment quality characteristics of the household water storage tanks.

and bacteria establish themselves in association with the sediment material. Schreiber and Schoenen (1994) indicated that benthic rotifers outnumber the planktonic variety; they found ~ 9,000 organisms/L of sediment sample, with rotifers and threadworms predominant. In this study, the sediment material contained nematodes, protozoa, and rotifers, with predominance of nematodes (dead and alive). The presence of organic material and algae in the sediments probably enhanced the growth of these invertebrates and caused the increase in their numbers. This explanation was confirmed by the observation of nematodes in varying stages, clear evidence that the organisms reproduced in the drinking water and household water storage tanks and did not merely survive coincidentally in the supply system. Levy et al (1986) indicated that invertebrates can engulf bacteria and permit their transport and resistance to chlorine in the water and correspondingly increase the public health significance of the presence of these organisms in drinking water.

Tank material affects bacterial diversity, sediment characteristics. No significant differences in HPCs were found among the different household tanks used in this study. Cluster analysis, however, indicated a similarity between the bacterial taxa found in the two cast-iron tanks' water and those found in the fiberglass and polyethylene tanks' water. This difference in bacterial diversity may be because of the higher temperatures and substrate availability in the fiberglass and polyethylene tanks, which provide a preferential environment for the growth of some bacteria, e.g., *Aeromonas*. The bacterial sediment characteristics of the household tanks were similar, with predominance of *Pseudomonas* spp. in all the tanks. The biological content of sediment was also similar in the four household tanks, with predominance of ciliate protozoa in the fiberglass tanks, where the sediment concentration was higher

and consequently the organic material may have enhanced the breeding and growth of these organisms.

This study raises questions about the validity of monitoring programs because after-treatment water quality may change considerably in the distribution system and in household tanks. Countries in which household water storage tanks are in wide use should upgrade their water quality monitoring systems to involve water quality after storage for the nonflow period. Consumers need to be aware of the "expiration date" of their water so that they can take remedial action to minimize any potential risk imposed by the increased bacteria in their water. Point-of-use ultraviolet water treatment devices at the tap are one option that should be considered to avoid health risks posed by microbial growth in household tanks. Another strategy calls for secondary chlorination in the household tanks. Regular (i.e., annual) cleaning regimes for tanks are also strongly recommended.

Bacterial Genera/Species	All Tanks %	Cast-iron 1 %	Cast-iron 2 %	Fiberglass %	Polyethylene %
Aeromonas	2.5		10		
Bacillus	2.5			10	
Micrococcus spp.	20		40	40	
Moraxella	2.5				10
Pseudomonas paucimobilis	2.5	10			
Pseudomonas vesicularis	10	10	10		20
Pseudomonas/Alcaligenes	7.5	10	10		10
Pseudomonas spp.	5.0	10			10
Yeast	2.5			10	
Unidentified isolates*	45	60	30	40	50

*Unidentified isolates consisted of 80% gram-negative nonfermentative bacteria, 10% gram-negative fermentative bacteria, and 10% bacterial colonies that lost or failed to grow on subsequent subculturing.

Table 4. Percentage of genera/species isolated and identified from the four household sediment samples.

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facsimile transmittal

To:	Alfred Lipshultz / Aquathin	Fax:	954-781-7336
From:	Arvind Patil	Date:	02/14/01
Re:	MIC Data for Microban B	Pages:	6
CC:			

☐ Urgent ☐ For Review ☐ Please Comment ☐ Please Reply ☐ Please Recycle

Notes:

Alfie:

Here is the data on Minimum Inhibitory Concentration s for Microban Additive B, that I promised.

MIC for *Legionella pneumophila* is added on page 2. It is 10 microgram per ml.

Regards

Arvind Patil

CONFIDENTIAL INFORMATION

ANTIMICROBIAL SPECTRUM OF MICROBAN® ADDITIVE "B"

Microorganisms	Code-No.*	Source**	Medium	M.I.C. mcg./ml.
GRAM-POSITIVE COCCI				
Staph. aureus	99	NCTC 7447	NA	0.01
Staph. aureus	122	NCTC 6571	NA	0.03
Staph. aureus	123	NCTC 6966	NA	0.1
Staph. aureus	322	ATCC 13709	NA	0.01
Staph. epidermidis	120	NCTC 7292	NA	0.1
Staph. epidermidis	742	INT	NA	0.03
Staph. lactis	461	NCTC 8340	NA	3
Micrococcus spp.	440	NCTC 2665	BHI-A	3
Sarcina lutea	119	NCTC 196	BHI-A	3
Streptococcus haemolyt. A	109	A	BHI-A	1
Streptococcus haemolyt. A	113	A	BHI-A	3
Streptococcus faecalis	114	NCTC 8619	BHI-A	10
Streptococcus faecalis	1071	ATCC 10541	BHI-A	3
Streptococcus pneumoniae	118	NCTC 7465	BHI-A	3
GRAM-NEGATIVE COCCI				
Branhamella catarrhalis	117	NCTC 3622	BA	33
GRAM POSITIVE RODS				
Lactobacillus Sp.	129	A	MACA	33
Lactobacillus casei	435	ATCC 7469	MACA	33
Lactobacillus arabinosus	485	CITM 706	MACA	33
Corynebact. kutscheri	547	A	BHI-A	3-100
Corynebact. diphtheriae	57	NCTC 3984	BHI-A	3
Listeria monocytogenes	60	NCTC 7973	EA	3
Erysipelothrix rhusiopath	58	NCTC 8163	EA	3
Bacillus subtilis	65	NCTC 8236	NA	0.1
Bacillus megatherium	63	A	NA	3
Bacillus cereus	134	A	NA	3
Bacillus cereus var. myc.	133	A	NA	3

CONFIDENTIAL INFORMATION

ANTIMICROBIAL SPECTRUM OF MICROBAN® ADDITIVE "B"

Microorganisms	Code-No.*	Source**	Medium	M.I.C. mcg./ml.
MYCOBACTERIA				
Mycobacterium tubercul.	203	A	YA	100
Mycobacterium smegmatis	140	CITM 78	BHI-A	1-3
Mycobacterium phlei	199	A	BHI-A	0.3
PROCARYOTIC ORGANISMS				
Mycoplasma pneumoniae	800	A	PPLO-A.	1000
ANAEROBIC ORGANISMS (see Actinomyces also)				
Clostridium botulinum	66	NCTC 3805	EA	3
Clostridium histolyticum	69	NCTC 503	EA	3
Clostridium welchii	72	NCTC 8359	EA	10
Clostridium sporogenes	195	A	EA	10
Clostridium butyricum	67	NCTC 7423	EA	10
Clostridium tetani	71	NCTC 9571	EA	3
ACTINOMYCELTALES				
Nocardia asteroides	229	NCTC 6761	BHI-A	1-3
Nocardia brasiliensis	676	A	BHI-A	1-3
Actinomyces israelii	694	NCTC 8047	NA	1-10
Actinomyces bovis	605	A	NA	1
Actinomyces naeslundii	603	A	NA	1
Actinobacillus israelii	602	A	NA	1
GRAM-NEGATIVE RODS				
Alcaligenes sp.	44	A	NA	100
Achromobacter sp.	304	A	NA	0.3-3
Escherichia coli	5	NCTC 8196	NA	0.03-0.30
Citrobacter freundii	767	A	NA	3->100

CONFIDENTIAL INFORMATION

ANTIMICROBIAL SPECTRUM OF MICROBAN® ADDITIVE "B"

Microorganisms	Code-No.*	Source**	Medium	M.I.C. mcg./ml.
Enterobacter cloacae	329	NCTC 8155	NA	0.3
Enterobacter aerogenes	242	CITM 413	NA	1-3
Klebsiella pneumonia	23	NCTC 8172	NA	0.3
Klebsiella sp.	243	INT	NA	1
Serratia marcescens	343	A	NA	>100
Proteus vulgaris (Neotype)	872	NCTC 4175	NA	0.01-0.3
Proteus mirabilis	316	A	NA	0.1-0.3
Proteus rettgeri	856	A	NA	33->1000
Morganella morganii	855	A	NA	10
Salmonella enteritidis	77	INT	NA	0.1-0.3
Salmonella typhimurium	661	A	NA	0.1-0.3
Salmonella typhi	570	NCTC 786	NA	0.1-0.3
Salmonella spp.	28	NCTC 5322	NA	0.1-0.3
Shigella flexneri	33	NCTC 8192	NA	0.1-0.3
Shigella sonnei	36	NCTC 7240	NA	0.1
Shigella dysenteriae	32	NCTC 2249	NA	0.1
Pseudomonas aeruginosa	25	NCTC 1999	NA	>100->1000
Pseudomonas fluorescens	26	NCTC 4755	NA	>100
Aeromonas sp.	375	A	NA	10
Pseudomonas mallei	954	NCTC 10230	NA	0.3
Pseudomonas pseudomallei	949	NCIB 9674	NA	1
Moraxella lacunata	552	A	NA	0.01
Moraxella glucidolytica	553	A	NA	0.3
Acinetobacter calcoaceticus	562	A	NA	0.1
Pasteurella multocida	46	NCTC 948	NA	0.1
Yersinia pseudotuberc	392	INT	NA	10
Haemophilus influenzae	921	A	NA	33
Brucella abortus	37	NCTC 8226	Br.A.A.	0.1
Brucella melitenis	41	A	Br.A.A.	0.1-1
Brucella suis	43	A	Br.A.A.	0.03
Legionella pneumophila				10

CONFIDENTIAL INFORMATION

ANTIMICROBIAL SPECTRUM OF MICROBAN® ADDITIVE "B"

Microorganisms	Code-No.*	Source**	Medium	M.I.C. mcg./ml.
		VIBRIO		
Vibrio cholerae	877	A	NA	10
Vibrio eltor	874	A	NA	10
		FUNGI & YEASTS		
Streptomyces sp.	682	A	BHI-A	1-3
Micromonospora chalcea	677	A	BHI-A	0.1
Aspergillus fumigatus	76	ATCC 9197	SMA	10
Blastomyces dermatitidis	382	ATCC 10225	SMA	3
Candida albicans	75	A	NA	3-33
Candida guillermondi	334	A	SMA	33
Candida parakrusei	647	A	SMA	3
Candida parapsilosis	332	A	SMA	33
Candida pulcherrima	643	A	SMA	33
Candida Stellatoidea	645	A	SMA	10
Candida tropicalis	644	A	SMA	10
Candida utilis	482	A	SMA	33
Cyrptococcus neoformans	646	A	SMA	10
Epidermonphyton floccosum	327	ATCC 10227	SMA	1-10
Hansenula anomala	483	A	SMA	10
Keratinomyces ajelloi	466	A	SMA	10
Microsporum canis	240	ATCC 10214	SMA	1-10
Microsporum cookei	406	A	SMA	33
Microsporum nanum	408	A	SMA	10
Microsporum gypseum	413	A	SMA	10
Neurospora sitophila	86	A	SMA	0.1
Penicillium egyptiacum	431	A	SMA	33
Pichia farinosa	642	A	SMA	33
Peidraia hortai	464	A	SMA	10
Rhodotorula mucilaginosa	331	A	SMA	33
Schizosaccharomyces pombe	651	A	SMA	3
Sporotrichum schenckii	83	ATCC 10212	SMA	3-10

CONFIDENTIAL INFORMATION

ANTIMICROBIAL SPECTRUM OF MICROBAN® ADDITIVE "B"

Microorganisms	Code-No.*	Source**	Medium	M.I.C. mcg./ml.
Trichophyton mentagrophytes	84	ATCC 9533	SMA	1
Trichophyton rubrum	351	A	SMA	10
Trichophyton tonsurans	396	A	SMA	10
Trichophyton concentricum	395	A	SMA	10
Trichophyton quinckeanum	397	A	SMA	10
Trichophyton schoenleinii	398	A	SMA	3
Trichophyton terrestre	410	A	SMA	10
Trichosporon cutaneum	333	A	SMA	10
Torulopsis dattila	336	A	SMA	10
Torulopsis glabrata	588	A	SMA	-
Troulopsis famata	338	A	SMA	10
Trichomonas foetus	837	A	ACM	100

* Number of internal strain collection.
**A = from outside institutes.

ATCC = American Type Culture Collection
NCTC = National Collection of Type Cultures (London)
CITM = Centre Intern. de Distribution de Souches (Sausanne)
NCIB = National Collection of Industrial Bacteria (U.K.)
INT = Internal collection

CONFIDENTIAL INFORMATION

ANTIMICROBIAL SPECTRUM OF MICROBAN® ADDITIVE "B"

MATERIAL AND METHODS

Micro-organisms. The micro-organisms tested were obtained from internationally known collections, of from varous clinics and institutes. Some strains were isolated in our own laboratories.

Nutrient media. The minimum inhibitory concentrations were determined in nutrient media generally containing between 1.5% and 2% agar, details of which are listed below. The particular medium used is always indicated together with the results.

Abbre-vation	Medium	Composition of Supplier		
NA	Nutrient agar	Bacto peptone (Difco No. B 118)	10g	
		Na Cl	3g	
		$Na_2HPO_4 \cdot 12\ H_2O$	2g	
		Liebig beef extract	10g	
		Bacto agar	20g	
		water, sufficient to produce	1000ml	
BA	Blood agar	NA + 10% (v/v) preserved human blood		
MACA	Micro assay cul. agar	Difco No. B 319		
BHI-A	Brain-heart inf. agar	Difco No. B 37		
EA	Eugon agar	BBL 01-265		
Br. A.A.	Brucella agar "Albumi"	Albimi Laboratories Inc., N.Y.		
YA	Youmans' agar	l-asparagine	5g	
		KH_2PO_4	5g	basal medium
		K_2SO_4	0.5g	
		water	1000 ml	
		Basal medium	270ml	sterilize and add 30 ml
		Noble agar	4.5g	bovine serum
PPLO-A	PPLO agar	BHI-A + 15% calf serum		
SMA	Sabouraud maltose agar	Difco No. B 110		
ACM	AC medium	Difco No. B 316 + 5% bovine serum		

NEW
INSERTS

LABORATORIO QUIMICO LAMBDA

Tels.: 286-1168 / 226-4462 • Fax:(506) 226-4462 • Apartado 877-1011 San José, Costa Rica
e.mail : lambda@sol.racsa.co.cr.

RESULTADO DE ANALISIS # 66,939

---RESULTADO DE ANALISIS BACTERIOLOGICO---

FECHA: 5 DE ENERO DEL 2001. **SOLICITANTE:** GRANJA AVICOLA LOS POLLITOS,SRL

ATENCION: COFASC.

REFERENCIA: MUESTRAS DE AGUA, RECIBIDAS POR EL LABORATORIO QUIMICO LAMBDA EL DIA 3 DE ENERO DEL 2001.

MUESTRA	RECUENTO TOTAL AEROBIO (U.F.C./dL)	COLIFORMES TOTALES (U.F.C./dL)	COLIFORMES FECALES (U.F.C./dL)
AGUA DESPUES DE FILTRO DESINFECTANTE	0	N.M.P. < 2	N.M.P. < 2
AGUA ANTES DE FILTRO DESINFEC.	87000	N.M.P. $6,4 \times 10^4$	N.M.P. < 2

OBSERVACIONES:

- **N.M.P.:** NUMERO MAS PROBABLE.
- METODOS ANALITICOS: STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER, 19TH EDITION, 1995.
- CONDICIONES PARA AGUA POTABLE: RECUENTO TOTAL MENOR A 1000, COLIFORMES TOTALES MENORES A 2 Y COLIFORMES FECALES MENORES A 2.
- LA MUESTRA DESPUES DEL FILTRO SE CONSIDERA MICROBIOLOGICAMENTE POTABLE, PERO LA MUESTRA ANTES DEL FILTRO SE CONSIDERA MICROBIOLOGICAMENTE NO-POTABLE.
- CODIGO LAMBDA: 6605A-1-2.

AQUASHIELD ANALYSIS

DESPUES = AFTER
ANTES = BEFORE

LABORATORIO QUIMICO LAMBDA
Teléfonos: 286-1168 - 226-4462
Apdo. 877-1011 San José, Costa Rica

Dra. ILEANA VEGA
COD. 173

NOTA:
Refiérase al número de este resultado para cualquier consulta.

SAENZ RENAULD
LABORATORIOS CLINICOS

DR. GERMAN F. SAENZ RENAULD, MQC. HEMAT.
DRA. CECILIA BEIRUTE PERALTA, MQC.

DR. RICARDO SAENZ BEIRUTE, MQC.
DR. JORGE FONSECA GONZALEZ, MQC. INM.

NOMBRE: Sra. COFACS FECHA: 16/02/2001

MEDICO: REF.:

ANALISIS BACTERIOLOGICO DE AGUA

AGUA DE _____ = Antes de Filtro No. 1

ASPECTO _____ = Transparente

OLOR _____ = Normal

SEDIMENTO AL FRESCO (PROTOZOARIOS) _____ = Negativo

SEDIMENTO ORGANI(X) _____ = Escaso

NUMERO MAS PROB. DE COLIFORMES (37°C) = 75 / dL (*)

NUMERO MAS PROB. DE COLIFORMES(44,5°C) = 4 / dL (**)

Recuento directo total _____ = > 1000 col./mL

(*) Corresponde a coliformes totales .

(**) Corresponde a coliformes fecales .

MQC
073-097-408-745-1066

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DR. JORGE FONSECA GONZALEZ, MQC. INM.

NOMBRE: Sra. COFACS

FECHA: 16/02/2001

MEDICO:

REF.:

ANALISIS BACTERIOLOGICO DE AGUA

AGUA DE_____ = Después de filtro I

ASPECTO_____ = Transparente

OLOR_____ = Normal

SEDIMENTO AL FRESCO (PROTOZOARIOS)_____ = Negativo

SEDIMENTO ORGANICO_____ = Escaso

NUMERO MAS PROB. DE COLIFORMES (37°C)_____ = < 1 / dL (*)

NUMERO MAS PROB. DE COLIFORMES(44,5°C)_____ = < 1 / dL (**)

Recuento directo total_____ = 0 col / mL (***)

(*) Corresponde a coliformes totales .

(**) Corresponde a coliformes fecales .

(***) Este recuento corresponde al total de bacterias de la muestra analizada.

MQC
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HOSPITAL Tel.: 222-1050 Fax: 221-4089
PASEO COLON Tel.: 223-0061 Fax: 256-3610
ROHRMOSER Telfax: 296-2289
ESCAZU Tel.: 289-5323 Fax: 289-7828
CARTAGO (ASOC) Tel.: 551-0338 Fax: 591-2997

Un laboratorio de confianza cerca de Usted!

fax transmission

To : Aquathin	**Fax :** 954 781-7336
At. : Alfred Lipshultz	**Date :** 19-04-2001
From : Christian Vieli	**Pages :** 2

Dear Alfie,

Please find attached the analisis results from the chicken farm. First row is filtered water and the second is not filtered.

I should get the PO these days for 27 or 54 AquaShields.

Thank you and best regards,

Christian Vieli

AMITEK S.A. Casilla 217 Correo 12, Santiago de Chile, phone 562 / 275-1124 fax 562 / 275-3286
www.amitek.cl chvieli@amitek.cl

SERVICIOS DE MANTENCION LTDA.
LABORATORIO BIOLOGICO

RP2603A

ORIGEN MUESTRA : MELTIL

FECHA INGRESO : 23-mar-01
FECHA INFORME : 26-mar-01

TIPO MUESTRA : AGUAS

CLAVE				TIPO MUESTRA	RCTO TOTAL UFC* ml	COLIF. TOTAL UFC* ml	COLIF. FECAL UFC* ml
RP	22	3	66	muestra 1 filtrada /tranque sur	<25	0	0
RP	22	3	67	muestra 2 no filtrada/tranque sur	10000	0	0

HUGO CHAPARRO M.

SRS. J.FREIRE
C.C. C.REAL-J.R.VILLAR-C.ESCALONA

AQUATHIN

Publics Plaza N.19, Canape Vert - Tel. 45-4250

MARCH 18, 2000

ALFRED LIPSHULTZ
AQUATHIN
950 SOUTH ANDREWS AVENUE
POMPANO BEACH, FLORIDA. 33069

DEAR ALFIE:

FOR THE PAST MONTH, THE MUCKSUCKER FILTER HAS BEEN INSTALLED IN A POLYETHYLENE TANK IN OUR OFFICE IN HAITI. WATER FROM THE MAIN CISTERN WAS PUMPED PERIODICALLY TO THE 200 GALLONS TANK, AND TWICE DURING THAT TIME INTERVAL WATER SAMPLES WERE TAKEN TO ASSESS THE PRESENCE OR ABSENCE OF TOTAL COLIFORM. I AM PLEASED TO REPORT THAT BOTH TEST DID NOT CONFIRM THE PRESENCE OF TOTAL COLIFORM.

THE PRINCIPLE FOR THE MICROBIOLOGICAL TEST (COLILERT) THAT WAS USED IS BASED ON THE "DEFINED SUBSTRATE TECHNOLOGY" (DST) FOR PRESENCE OR ABSENCE OF TOTAL COLIFORM.

ADDITIONAL TEST RESULTS WILL BE PROVIDED TO YOU SHORTLY AS THEY BECOME AVAILABLE.

BEST REGARDS,

PIERRE A. SAJOUS

AQUATHIN

Publics Plaza N.19, Canape Vert - Tel. 45-4250

MAY 26, 2000

ALFRED LIPSHULTZ
AQUATHIN
950 SOUTH ANDREWS AVENUE
POMPANO BEACH, FLORIDA. 33069

DEAR ALFIE:

THE MICROBIOLOGICAL TEST PERFORMED LAST MONTH ON THE WATER SAMPLE COLLECTED FROM THE 200 GALLONS POLYETHYLENE TANK CONFIRMED AGAIN THE ABSENCE OF TOTAL COLIFORM. I AM ALSO PLEASED TO REPORT THAT AFTER THREE MONTHS OF OPERATION, THE WATER IN THE TANK DID NOT SHOW ANY VISIBLE SIGN OF ALGAL GROWTH.

BEST REGARDS,

PIERRE A. SAJOUS

LabHouse
York House, Epsom Downs Office Park, Sloane Street, Epsom Downs, Bryanston 2152
P.O. Box 344, Cramerview 2060, South Africa
Tel: +27 11 463-5760
Fax: +27 11 463-7330
E-mail: labhouse@yebo.co.za

AQUATHIN
PO BOX 2622
RIVONIA
2128

ORIGINAL

Tel: 011 468 2768 **Fax: 011 468 3124**

Attention: Gordon Bastiaans

LABORATORY TEST REPORT

Sample: **Water filter system (Microban) inoculated with *Vibrio cholera* (Before filtration)**
Sample number: LH20011014A
Date: 14/10/2001
Date completed: 21/10/2001

LABORATORY TEST REPORT

Test	Method	Specifications	Result
Total Plate Count for *Vibrio Cholera*	SABS ISO 4833:1991	-	2.5 X 10^3 cfu/ml
Vibrio cholera detection	SABS 1196:1992	-	Detected

TRACEY-LEE BOTES
TECHNICAL MANAGER

LabHouse
York House, Epsom Downs Office Park, Sloane Street, Epsom Downs, Bryanston 2152
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E-mail: labhouse@yebo.co.za

AQUATHIN
PO BOX 2622
RIVONIA
2128

ORIGINAL

Tel: 011 468 2768 **Fax: 011 468 3124**

Attention: Gordon Bastiaans

LABORATORY TEST REPORT

Sample: **Water filter system (Microban) inoculated with *Vibrio cholera* (After filtration day 1)**
Sample number: LH20011014B
Date: 15/10/2001
Date completed: 21/10/2001

LABORATORY TEST REPORT

Test	Method	Specifications	Result
Total Plate Count for *Vibrio Cholera*	SABS ISO 4833:1991	-	<1.0 X 10^1 cfu/ml
Vibrio cholera detection	SABS 1196:1992	-	Not Detected

aquathin

DAY ONE
AFTER
100+AS

TRACEY-LEE BOTES
TECHNICAL MANAGER

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PO BOX 2622
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2128

ORIGINAL

Tel: 011 468 2768 **Fax: 011 468 3124**

Attention: Gordon Bastiaans

LABORATORY TEST REPORT

Sample: **Water filter system (Microban) inoculated with *Vibrio cholera* (After filtration day 2)**
Sample number: LH20011014C
Date: 15/10/2001
Date completed: 21/10/2001

LABORATORY TEST REPORT

Test	Method	Specifications	Result
Total Plate Count for *Vibrio Cholera*	SABS ISO 4833:1991	-	$<1.0 \times 10^1$ cfu/ml
Vibrio cholera detection	SABS 1196:1992	-	Not Detected

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DAY TWO
AFTER
100+AS

TRACEY-LEE BOTES
TECHNICAL MANAGER 083 634 0088