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A MICROBIOLOGAL SURROGATE FOR EVALUATING TREATMENT EFFICIENCY

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INTRODUCTION

In this study we report on the use of a microbial surrogate system which can be used to evaluate the efficiency of various unit processes used in drinking water treatment for the removal of microbial contaminants. The proposed procedure uses Gram-positive, mesophilic, aerobic spore-forming bacteria as the surrogate organisms. These bacteria do not pose a public health threat and are naturally occurring in most surface water supplies. The aerobic spore-formers are easy to culture and are present throughout the treatment train. This group of organisms consists primarily of species of the genus *Bacillus*. These organisms form endospores which are ellipsoidal to spherical in shape and measure on average approximately 0.5 X 1.0 X 1.5 micrometers, and are environmentally resistant. Like pathogenic *Giardia* cysts and *Cryptosporidium* oocysts endospores of aerobic bacilli may be found far into the treatment train.

MATERIALS & METHODS

Both naturally occurring aerobic spore-forming bacteria and pure cultures of *Bacillus subtilis* spores were used in this study. *B. subtilis* spores were purchased from a commercial laboratory (Raven Biological Laboratories, Omaha, Nebraska 68106). The protocol for the enumeration of the aerobic spores was a modification of a procedure used for the detection of *Bacillus* spores in raw milk (1). The following equipment is used in the protocol:

- Sterile Erlenmeyer flasks with screw-caps or sterile bottles with closures.
- Thermostatically controlled water bath with shaker for constant agitation
- Thermometer
- Sterile pipettes
- 60 x 15 mm Petri dishes with loose lids
- 47 mm, 0.45 μm porosity membranes
- Membrane filtration apparatus
- Incubator, 35°C

The medium used for growing the bacteria consisted of nutrient agar plus the dye trypan blue. The dye was added to aid in the visualization of the colonies.

The formulation for the medium is:

- Nutrient Agar
  - Peptone 5 g
  - Beef extract 3 g
  - Agar 15 g
- Trypan blue dye 0.015 g
- Distilled water 1,000 ml

  Heat to boiling dissolve completely.
  Sterilize in the autoclave for 15 minutes at 15 psi (121°C)
  pH 6.8 ± 0.2 at 25°C

The spore-forming bacteria were enumerated using the membrane filter method. The following steps outline the experimental procedure:

- Place sample in sterile flask and cover with cap
- Prepare pilot flask with same volume as sample and thermometer
- Place sample flask(s) and pilot flask in water bath at 82°C and agitate throughout heat treatment
- Lower temperature of bath to 80°C when flask(s) reaches 79°C
- When contents of flask(s) reach 80°C, keep samples in bath for an additional 12 min.
- Immediately cool sample flask(s) in ice bath
- Membrane filter (0.45 μ m pore size) appropriate volumes and place membrane on medium
- Incubate plates 20-22 hr at 35°C
- Count colonies

Heating the sample to 80°C inactivates vegetative bacterial cells but does not inactivate spores. During aerobic incubation at 35°C the spores germinate and are counted as bacterial colonies.

Total coliform bacteria were enumerated by the membrane filter procedure using m-Endo LES agar, and heterotrophic plate count (HPC) analyses were conducted by the spread plate procedure using R2A agar with incubation of plates at 28°C for 5-7 days. Cultures of *B. subtilis* spores were used in the bench-scale coagulation studies (jar tests). Standard procedures were used to conduct the jar test experiments (3). An electronic particle counter, HIAC/ROYCO, model 9064 (Pacific Scientific Co., Montclair, Calif.) was used for particle sizing and counting analysis.

RESULTS & DISCUSSION

Levels of indigenous aerobic spores found in the Ohio River over a ten month period are listed in Table 1. These results are typical of the numbers of aerobic spore-formers which would normally be encountered in a surface water supply.

Table 1.

Levels of Indigenous Spores

in

Ohio River Water

1994

Month	CFU/100 ml
January	7,500
February	31,000
March	11,000
April	27,000
May	5,800
June	3,700
July	3,900
August	310,000
September	2,600
October	1,800

Table 2 lists the mean log removals of several microbial parameters and total particles (>1 μm) and particles in the size range of 3.1-7 μm in a pilot conventional drinking water treatment plant sampled daily over seven days. This table lists the log removals achieved through the settling and filtration processes. The spores were the only microbial parameter which could be found throughout the treatment train and their removals closely paralleled both total particle counts and counts in the 3.1-7 μm size range. Table 2 also indicates that the spores, like *Giardia* and *Cryptosporidium*, are much more resistant than coliforms or HPC to chlorination.

Log removals of spores and particles in a full-scale water treatment plant which utilizes Ohio River water are listed in Table 3. These data collected over three seasons again show the parallel between particle counts and levels of indigenous spores.

CONCLUSIONS

Indigenous aerobic bacillus spores can serve as surrogates for particle removal and potentially for *Giardia* and *Cryptosporidium* removals. Generally, optimal removals were achieved with spores when optimal removal efficiencies were achieved for turbidity and particle removals. The aerobic spore forming bacteria are ubiquitous in nature and thus can be used to evaluate treatment without adding potential surrogates to the source water. Since these bacteria and their spores are primarily soil organisms their numbers increase with increasing amounts of run-off. Unlike the general heterotrophic plate count parameter, these organisms would not propagate in the treatment plant (e.g. as the result of increased AOC after ozonation) and are more resistant to disinfection. Also, unlike inanimate particles, which are counted by electronic particle counters, the spores would not be created or broken up while passing through various unit processes. While these organisms have no public health significance and are not indicators of fecal contamination, they may serve as useful indicators of treatment plant efficiency.

REFERENCES

1. American Public Health Assoc. 1985. Standard Methods for the Examination Dairy Products. 15th ed. Am. Pub. Health Assoc. Washington, D.C.

2. American Public Health Assoc. 1992. Standard Methods for the Examination of Water and Wastewater. 18th ed. Am. Pub. Health Assoc. Washington, D.C.

Table 2.

Removals During Pilot Study

Parameter	Log_{10} Removal Settled	Filtered Chlorinated
Total coliforms	0.47	>1.79*
HPC	0.38	>4.58*
Spores	0.85	2.11
3.1-7 μm particles	1.11	2.10
Total particles	0.91	1.70

* Below detection limit:
Total coliforms 1 cfu/100 ml
HPC 1 cfu/ml

Table 3.

Seasonal Removal - Ohio WTP

	Log_{10} Removal from Raw Water		
	Spores	3-6 μm Particles	Total Particles
Winter			
settled	1.35	1.62	1.37
finished	2.86	3.52	3.26
Spring			
settled	1.51	1.08	1.47
finished	2.80	2.22	2.50
Summer			
settled	2.11	0.61	0.73
finished	2.08	2.09	1.97