Rapid Detection of Microorganisms in Aerospace Water Systems

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As few as 100 to 300 bacterial cells can be detected in less than one minute by a method being developed for use aboard manned spacecraft. Spacecraft water supplies, particularly those in which wastewater is recycled for drinking purposes, require bacteriological monitoring for protection of the crew. Standard microbiological assay methods cannot produce results rapidly enough for quality control purposes. Accordingly, a new, rapid, highly sensitive method for the detection of bacteria in water is being developed. The procedure utilizes the firefly bioluminescence assay for adenosinetriphosphate (ATP). ATP has been found in all living organisms thus far examined. If sterile water supplies are required for spacecraft, the total bacteria count would be the best quality index. The ATP content per cell for a number of species of bacteria, algae, fungi and protozoa is reported along with results obtained from selected species in different growth stages. The mechanics of the technique and the design of the bioluminescence detector are described. The development of spacecraft instrumentation to utilize the method for monitoring the safety of drinking water for aerospace crews is discussed.

UTILIZING the bioluminescent reaction of the firefly, a method is being developed to detect microbial contamination of manned spacecraft water supplies. The current status of the method permits the detection of several hundred cells, or less, within one minute. Progress to date promises a real time, in-flight means for protecting astronaut crews against the hazards of ingesting contaminated water. Such monitoring will be particularly important aboard spacecraft in which liquid wastes are recycled.

Currently used standard methods for the examination of drinking water are inadequate for use aboard spacecraft for several reasons. The elapsed time of the assays, 24 to 96 hours, is too long to permit corrective action to be taken prior to consumption of the water. Furthermore, the criterion used by the U. S. Public Health Service for the determination of the bacterial quality of drinking water relates only to the coliform group of organisms. These organisms, of fecal origin, are an index of sewage contamination. The sources of contamination aboard the spacecraft are not all directly of sewage origin and thus conveniently tagged with coliform organisms. Finally, standard methods would require the continuous use of a 35°C incubator aboard the spacecraft with concomitant heavy requirements for power. With careful control, it seems possible and desirable to maintain spacecraft water supplies in sterile condition.[5] The new method detects all microorganisms and, thus, is compatible with this concept.

METHOD

The basis of the method[3] is a novel association of two biochemical findings: a. Adenosinetriphosphate (ATP) is specifically required in the firefly bioluminescence reaction[4]; b. ATP is ubiquitous in all living cells.[1] The following steps comprise the assay technique: 1, preparation of firefly lantern reaction mixture, 2, placement of aliquot of reaction mixture into cuvette in light sensing instrument, 3, extraction of microbial ATP from suspect water sample 4, injection of aliquot of sample extract into cuvette containing firefly lantern reaction mixture, and 5, measurement of peak light intensity produced by reaction.

The peak light intensity is directly proportional to

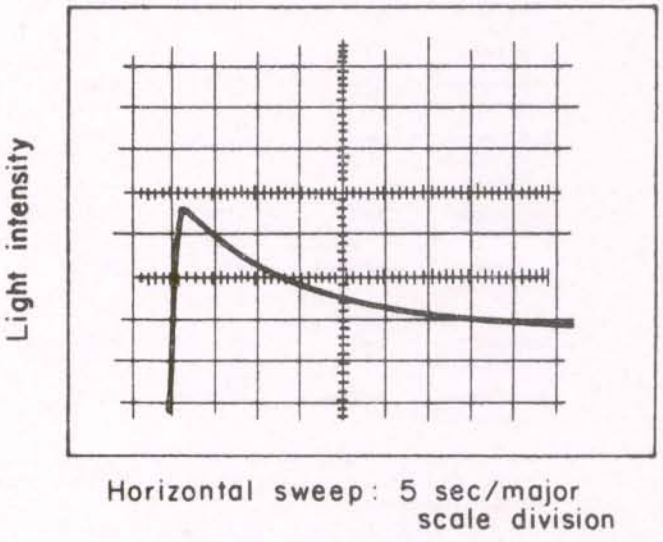

Fig. 1. Typical response of microbial ATP in firefly bioluminescent reaction.

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the quantity of ATP extracted from cells present in the water sample. Step 1 can be performed in advance of the test and the firefly enzyme reaction mixture lyophilized and stored. Steps 2 through 5 can be executed in less than one minute. Standard curves made with aqueous solution of stock ATP provide the required calibration for the assay. Biochemical and instrumentation advances have made possible the detecion of as little as 10^{-7} micrograms of ATP, equivalent to the ATP contained in approximately 100 cells of *E. coli* or one *Tetrahymena* cell.

RESULTS

When the light produced by the reaction is measured on an oscilloscope, a curve typified by Figure 1 is generated. The peak rise occurs in less than one second after initiation of the reaction. Figure 2 demonstrates the linearity of the response to ATP standards in a range spanning several orders of magnitude.

At the time of writing, 10 of 12 selected species of microorganisms, grown in pure cultures, have been assayed for ATP content by the bioluminescent method. The selection of the test organisms, shown in Table I, was mainly based on challenging the systems with a wide variety of metabolically different organisms in order to evaluate the nonspecificity of the technique. Secondly, the group of organisms included types that

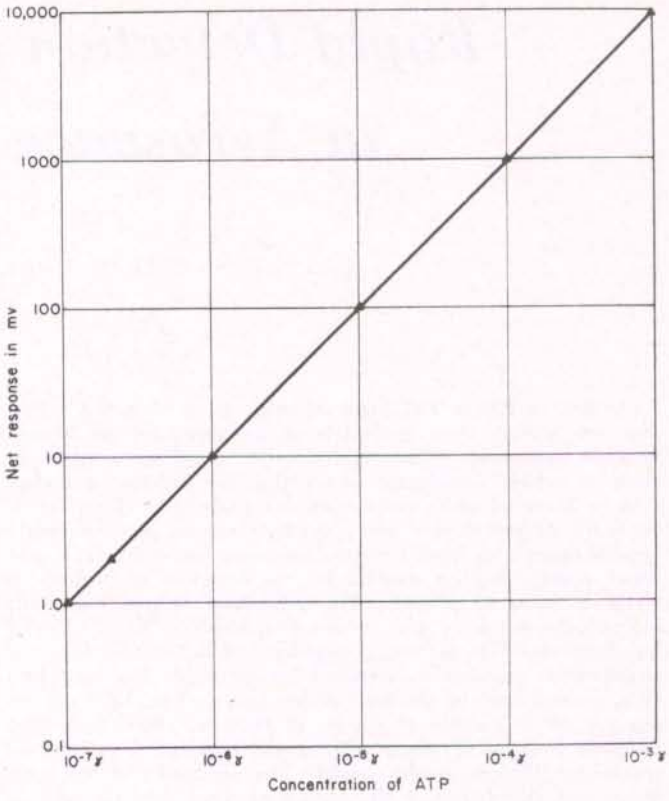

Fig. 2. Standard ATP curve on bioluminescent detector.

TABLE I. CELL CHARACTERISTICS AND CULTURE SYSTEMS FOR TEST ORGANISMS

			Preparation of test cultures								
Test organisms	Morphology	Size (μ)	Media*	Inocula ture (hr)	Test cul- (hr)	Approx- imate density (cells /ml)	Growth condi- tions	Cell count** Plate	Chamber	Oxygen require- ments	Energy source
Escherichia coli (a)	Coccoid to rods	0.5 × 1-2	TSB	24	5	10^9	37° C (shaker)	TSA	PH	Facultative anaerobic	Hetero- troph
Corynebac- terium striatum (a)	Pleomorphic rods	0.3 0.5 × 2.0-3.0	TSB	24	5	10^7	37° C (shaker)	TSA	PH	Aerobic	Hetero- troph
Bacillus subtilis (a)	Rods	0.7-0.8 × 2.0-3.0	TSB	24	5	10^9	37° C (shaker)	TSA	PH	Aerobic	Hetero- troph
Clostridium sporo- genes (a)	Rods & spores	0.6-0.8 × 3-7	FTG	24	24	10^9	37° C (static)	FTGA	PH	Anaerobic	Hetero- troph
Streptococcus salivarius (a)	Cocci	0.8-1	TSB	24	5	10^{10}	37° C (shaker)	TSA	PH	Facultative anaerobic	Hetero- troph
Staphylococ- cus epider- midis (a)	Cocci	0.5 0.6	TSB	24	5	10^8	37° C (shaker)	TSA	PH	Facultative anaerobic	Hetero- troph
Pseudomonas fluorescens (a)	Rods	0.3 0.5 × 1-1.8	TSB	24	6	10^7	25° C (shaker)	TSA	PH	Aerobic	Hetero- troph
Chlorella pyrenoidosa (b)	Spherical	4-6	NG	—	48	10^7	37° C (shaker)	—	P	Aerobic	Autotroph
Saccharomyces cerevisiae (c)	Oval	8-10 × 5-7	SDB	24	5	10^7	25° C (shaker)	SDA	PH	Facultative anaerobic	Hetero- troph
Tetrahymena pyriformis (d)	Pyriform	50 × 30	TB	—	72	10^6	25° C (shaker)	—	P	Aerobic	Hetero- troph

*TSB: Tryptic Soy Broth. NG: Nitrate Glucose Broth. SDB: Sabouraud Dextrose Broth. FTG: Fluid Thioglycollate Broth. TB: Tetrahymena Broth.

** TSA: Tryptic Soy Agar. FTGA: Fluid Thioglycollate Agar. SDA: Sabouraud Dextrose Agar. PH: Petroff-Hausser Chamber. P: Palmer Chamber.

a: bacteria b: algae c: fungi d: protozoa

TABLE II. SUMMARIZED RESULTS OF ATP RESPONSE BY LOWEST NUMBER OF CELLS TESTED AND AVERAGE ATP (γ) PER CELL

Test Organisms	Cell Size (μ)	Responsive minimum number of cells tested		ATP (γ)	Average ATP (γ) Per Cell*	
		Cell counts				
		Plate Count	Chamber Count		Plate Count	Chamber Count
Staphylococcus epidermidis	0.5–0.6	100	170	7.1×10^{-8}	4.1×10^{-10}	2.2×10^{-10}
Pseudomonas fluorescens	0.3–0.5×1–1.8	50	100	9.5×10^{-8}	1.6×10^{-9}	5.9×10^{-10}
Streptococcus salivarius	0.8–1	200	400	2.8×10^{-7}	1.1×10^{-9}	5.7×10^{-10}
Escherichia coli	0.5–1–2	300	270	2.6×10^{-7}	5.6×10^{-10}	5.9×10^{-10}
Bacillus subtilis	0.7–0.8×2–3	300	300	7.6×10^{-7}	3.1×10^{-9}	2.4×10^{-9}
Corynebacterium striatum	0.3–0.5×2–3	27	53	8.5×10^{-8}	9.7×10^{-9}	4.9×10^{-9}
Clostridium sporogenes	0.6–0.8×3–7	16	310	4.2×10^{-8}	2.5×10^{-9}	2.1×10^{-10}
Saccharomyces cerevisiae	8–10×5–7	4.7	27	5.1×10^{-7}	1.3×10^{-7}	2.8×10^{-8}
Chlorella pyrenoidosa	4–6	—	0.6	5.0×10^{-8}	—	5.0×10^{-8}
Tetrahymena pyriformis	30–50	—	0.6	1.5×10^{-6}	—	2.8×10^{-6}

*Average for all counts of 8-12 tests per organism.

could become spacecraft water supply contaminants. Optical density data were obtained with cultures of each species. This permitted rapid estimation of culture densities at the time of the tests so that dilutions could be made to approximate the desired numbers of cells. At least two dilutions of each species taken from four separate cultures were assayed. Plate and chamber counts were made for each assay in order to provide more exact information for correlating the amount of ATP detected with the numbers of cells actually assayed. Complete details and results of this one-year program are presented in a separate report.[2]

A high degree of sensitivity is demonstrated for the method. The Air Force desires the method to detect as few as 10-100 bacteria, fungi, algae and protozoa per ml. Table II lists the minimum number of cells contained in the aliquots tested, not the minimal detectable numbers which would, of course, be smaller. For the species tested, the Air Force requirements can readily be met although membrane filtration of 25 or 50 ml of the water to be tested may be necessary in some cases. Furthermore, the results indicate that by filtering large quantities, the method can effectively monitor the water for sterility.

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