Sewage Treatment by Controlled Eutrophication: Bacterial Study

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Several groups of bacteria were isolated and identified in an evaluation of the microbiological properties of a sewage treatment system involving the process of controlled eutrophication in a marine setting (J. G. Songer, N. M. Trieff, R. F. Smith, and D. Grajcer, 1974). Fecal coliforms, enterococci, Salmonellae, Shigellae, Vibrio parahaemolyticus, and Vibrio alginolyticus were studied at three stages of the treatment process. Significant reductions in fecal coliforms (\(P < 0.01\)) and enterococci (\(P < 0.01\)) were noted from raw sewage to effluent. Salmonellae and Shigellae were not detected at any stage, nor was V. parahaemolyticus. V. alginolyticus was isolated from the effluent only, reflecting the halophilic nature of the organism; low concentrations in raw sewage increased in the more saline effluent. Brine shrimp (Artemia salina), the herbivores in this system, were tested and found to have extremely low numbers of the organisms under study associated with them. Findings point further toward the use of this system as a combined mariculture-sewage treatment facility.

Many bodies of water are somewhat self-contained with regard to biological components. However, the metabolic rates and relative stability of the body of water are determined to a significant extent by the input of solar energy and especially by the rate of inflow of water and other materials from the watershed. Materials tend to accumulate when bodies of water are small or if the outflow is reduced. When man increases the input of materials to a system (particularly in the form of organic material, e.g., sewage and industrial wastes), the rapid accumulation that results may be destructive to the system. Natural eutrophication can be defined as aging, whereas cultural, or induced, eutrophication (enrichment) denotes organic pollution resulting from man's activities (2). Controlled eutrophication involves the artificial enrichment of a system and harvest of the nutrients as a crop at the desired trophic level (4).

A system similar to many aquaculture, la-gooning, or oxidation pond-type systems has been developed on the laboratory scale (5; M. McShan, N. M. Trieff, and D. Grajcer, 1974, J. Water Pollut. Contr. Fed., in press) involving the use of the marine alga Tetraselmis chui for controlled eutrophication of raw sewage. Algae grown from raw sewage are fed to brine shrimp, Artemia salina. Net products of the system are reported as: (i) brine shrimp for use as fish food or shrimp food; and (ii) a purified effluent.

Human feces and sewage wastes represent a major source of waterborne pathogens. Transmission of disease by way of contaminated water has been recognized as a possibility since Snow's work with cholera and the Broad Street pump. During the early 1900s, an important research topic was the transmission of human pathogens by fish and other aquatic organisms; more recent work on this subject has been rare. With increasing microbial contamination of water, increased consumption of fish, and more contact with the aquatic environment, the assumption that aquatic organisms cannot serve as disease vectors becomes a more hazardous one from a public health point of view (1).

Our research was designed to study bacterial pathogen flow through the proposed system of sewage treatment by using controlled eutrophication. On the basis of widely used standards for assessing the quality of food and water and also the marine nature of this system, the following bacterial groups were chosen for study: (i) fecal coliforms, (ii) the enterococci, (iii) the salmonellae and shigellae, and (iv) two halophilic vibrios of concern in the field of mariculture, V. parahaemolyticus and V. alginolyticus.

MATERIALS AND METHODS

A continuous-flow system (5) was used (Fig. 1). Four sets of samples were taken at three stages (raw...
sulfate stated, summative of the MPN. Blood media multiple-tube tubes made sheeprbase Cockeysville, months and subjected shrimp, tests. Enrichment cultures for V. parahaemolyticus and V. alginolyticus (in alkaline peptone broth from Difco Laboratories, Detroit, Mich.) were streaked to TCBS agar (BBL). Appearance of typical colonies in 24 h indicated the presence of vibrios. Suspect colonies were subjected to biochemical tests, again using the API-20 profile recognition system. NaCl (3%) was used as diluent. A correct profile confirmed the presence of V. parahaemolyticus (#4346106) or V. alginolyticus (#4146124).

RESULTS AND DISCUSSION

Results of testing for coliforms and enterococci are summarized in Table 1. As expected, a reduction in number of fecal coliforms was seen on passage of raw sewage through the system (raw sewage-effluent difference was significant at \( P < 0.01 \)). There were similar patterns for the enterococci (raw sewage-effluent difference was significant at \( P < 0.01 \)). Neither Salmonella, Shigella, nor V. parahaemolyticus was detected at any stage.

The detection of V. alginolyticus in three of four samples of the effluent was unexpected but not wholly unexplainable. The proximity of the sewage treatment plant to Galveston Bay is the most likely explanation for its presence in raw sewage (exceptionally high tides cause an occasional infiltration of seawater into raw sewage lines). It is doubtful that significant numbers of this organism would be present due to human intestinal discharge. The halophilic nature of V. alginolyticus is an important consideration here. Physiological stress due to the freshwater composition of sewage (as well as other cytotoxic factors) would keep the organism in small numbers, and hence it would remain undetected in raw sewage and in the algal culture. The brine shrimp tank represents a more favor-

![Schematic diagram](image)

**Fig. 1.** Raw sewage and Instant Ocean are pumped into the algal culture, displacing algae into the brine shrimp tank. An equal volume, the system's effluent, is pumped out of the brine shrimp tank.

sewage, algal culture, and effluent) over a period of 2 months and subjected to the various tests. For analysis of brine shrimp, 100 of them were collected and macerated, and the tissue homogenate was diluted with sterile distilled water to the volume needed for the tests. Data were analyzed by analysis of variance and the multiple-range test. Except where otherwise stated, incubation was at 37°C for 48 ± 3 h and all media were from Baltimore Biological Laboratories, Cockeysville, Md.

**Bacteriological methods.** A coliform most probable number (MPN)/100 ml was determined by the multiple-tube fermentation technique using lauryl sulfate broth. Transfers were made from positive tubes to EC broth; incubation of the transfers was at 44.5°C in a water bath. Growth and gas in 24 h indicated the presence of fecal coliforms and confirmed the MPN.

Azide dextrose broth served as a primary medium for enterococci yielding an MPN/100 ml. positives were transferred to ethyl violet azide broth. Turbidity and the appearance of a purple button constituted a positive test; streaks were made to Columbia CNA blood agar (composed of the Columbia CNA agar base containing colistin and nalidixic acid with 5% sheep blood added). Typical colonies were picked to Enterococcosel broth, where blackening of the medium in 24 ± 2 h gave a completed test and confirmation of the MPN.

Growth in Selenite-F broth was considered a presumptive test for Salmonella or Shigella; streaks were made to MacConkey agar. Typical colonies were picked to Kligler iron agar slants and incubated for 18 h. Cultures giving reactions typical of Salmonella or Shigella were subjected to biochemical testing in the API-20 profile recognition system (Analytab Products, Inc., New York, N.Y.). A correct profile confirmed the presence of Salmonella or Shigella.

**Table 1. Summary of coliform and enterococcus data**

| Material          | Coliforms | Enterococci |
|-------------------|-----------|-------------|
|                   | \( \bar{X} \) | SE \( a \)   | \( \bar{X} \) | SE \( a \)   |
| Raw sewage        | 7.138     | 0.627       | 4.251     | 0.081       |
| Algae culture     | 3.080     | 0.286       | 1.953     | 0.257       |
| Effluent          | 1.570     | 0.581       | 0.558     | 0.364       |
| Brine shrimp      | 0.571     | 0.344       | 0         | 0           |

\( a \) Mean of \( \log x \) values.

\( b \) SE, Standard error of the mean.

\( c \) Per 100 ml.

\( d \) Groups of 100.
able environment to the *Vibrio* (approximately 20 g/liter salinity and bottom sediments rich in organic matter), allowing its multiplication and subsequent detection of the organism in the effluent and brine shrimp. Instant Ocean, a synthetic marine mix which simulates sea water, and brine shrimp eggs were assayed for halophilic organisms and were found to contain no bacteria resembling *V. alginolyticus* or *V. parahaemolyticus*.

The results in testing of the brine shrimp for sewage-related organisms give further promise to the use of this system as a combined sewage treatment-mariculture facility. It is most likely that the few coliforms, enterococci, and vibrios found in the *Artemia* were associated with algae in their guts. Further study is needed to determine whether vibronic disease is produced in shrimp by feeding them brine shrimp from this system. Oysters, which have been used by others (3, 4, 6), are notorious for their ability to concentrate sewage-borne toxins, ranging from microorganisms to heavy metals. Further study of the brine shrimp produced here (e.g., with regard to pesticides, viruses, parasites, heavy metals, and so forth) is needed before their use as food for fish or shrimp can be considered positively safe.

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