

M-II1

RECOVERY OF INJURED FECAL COLIFORMS IN SEAWATER BY THE MOST-PROBABLE-NUMBER PROCEDURE (MPN) AND TWO MEMBRANE FILTRATION TECHNIQUES (MF) (M7-H-FC AND MT-7 AGAR MEDIA)

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Introduction

Two methods are specified by the E.E.C. for the microbiological analysis of recreational waters (1), the MPN method and the membrane filter techniques. None of the recommended methods specify resuscitation (2) and in seawater a number of environmental factors injure or kill coliform bacteria (3). As a result many investigators have proposed alternative MPN and MF methods (4)(2)(5).

Traditional MPN procedure was compared with two membrane filtration methods, a rapid 7 hour fecal coliform test (5) and a medium (mT-7 agar) designed to improve recoveries of injured fecal coliforms (4).

Materials and Methods

Water samples were collected in two sites on the beach of Puebla de Farnals (Valencia) during the period January-April 1986. The first site is next to a drain of fecal contaminated waters. Samples were prefiltered with 12 µm sterile membrane filters (Sartorius) to eliminate particles on suspension.

Microbial methods:

m7-h FC test.- samples (prefiltered) were filtered with 0.45 µm membrane filters (Millipore) and membrane filters were placed on m7-h FC medium. The plates were incubated for 7 to 7.25h at 41.5°C (5).

mT-7 agar method.- the resuscitation procedure consisted of filtration of two replicates of each sample dilution through membrane filters 0.45 µm (Millipore). mT-7 agar was used as the fecal coliform medium. The plates were preincubated for 8h at 37°C followed by incubation for an additional 12h at 44.5 °C (7).

MPN test.- fecal coliform procedure was conducted as defined in Standard Methods (6).

Confirmation of isolates.- at least 7 presumptive fecal coliforms from each MF technique were verified by inoculation into lauryl triptose broth (37°C for 48h) followed by transfer to EC broth (44.5°C for 24h) (5)(7).

Statistical analysis: statistical comparisons were made on logarithmically transformed data by the analysis of variance.

Results

Counts on MPN method were generally lower than on the other two methods in both sites. T-7 agar yielded the highest average for each sample point. The counts in site 1, between the three procedures shows greater differences which indicate that the quality of water had significant effect on the ability of media to support the production of fecal coliform population.

In site 1 the mean ratio m7-h count per 100ml/MPN per 100 ml ranged from 1-1.12, the mean ratio mT-7/m7-h ranged from 1-1.1 and the mean ratio mT-7/MPN ranged from 1.1-1.3. In site 2 the mean ratio between the three procedures were very similar to site 1. Verification rates for fecal coliform isolates on mT-7 agar averaged 85.7% whereas verification rates for m7-h averaged 80.5%. The analysis of variance revealed no significant differences between the three procedures in site 2 (P<0.05) but in site 1 the difference was highly significant (P<0.05) between the three techniques.

Discussion

The time and temperature of preincubation were found to be crucial to the optimal recovery of injured fecal coliforms in both membrane filter procedures (5)(7).

Verification rates for fecal coliform isolated on mT-7 agar averaged 85.7% for seawater samples whilst LeChevallier (7) averaged 89% for chlorinated and no chlorinated waters. This medium was found to produce consistently higher fecal coliform counts than the two other methods tested.

The m7-h FC can be used for the emergency examination of coastal waters with fecal contamination.

The 12 µm sterile prefiltration step of samples eliminates the interference due to particles on suspension when used the MF procedures.

Modified culture methods are required for recovery and enumeration of sublethally stressed organisms (2)(8).

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M-II2

SOME ASPECTS ABOUT MICROBIAL ACTIVITIES ON NITROGEN TRANSFORMATION IN CULLERA BAY

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Introduction

Cullera bay is a tourist village situated 40 km. far from Valencia which increases its population in a large number during summer periods. Seawater in Cullera bay is influenced by the contaminant discharge of Júcar river; this fact and the restrictive circulation of their waters causes a serious pollution problems. During the last years (due to the increase of pollution) nitrogen cycle has been intensely studied (1,2,3,4,5) principally in bays, estuaries and lagoons which receive many pollution sources. A great quantity of nitrogen compounds derived of urban wastes are present in these discharges. These waste water discharges may be monitored in order to the public health control but the amount of nutrients which they transport is not removed by the water treatment systems. Consequently, an eutrophication of receptor waters is produced.

In the present work ammonification and nitrification (autotrophic and heterotrophic paths) activities developed by microorganisms have been studied. Nitrites, nitrites and ammonia values of seawater have been also monitored. Statistical analysis with data obtained were calculated.

Materials and Methods.

Sampling: Water samples were collected at 3 points in Cullera bay, at surface and depth (5mts.) during the months of May, July, August and October.

Microbial Methods: Ammonification and Nitrification activities: MF-Technique.- Samples were filtered with a 0.45µ pore size filter (Sartorius SM111). Filters were inoculated into a flask with 100ml. of specific medium (6) and incubated for 3 days at 28°C for ammonification activities and for 18 days at 28°C in the case of nitrification activities.

Chemical Determinations: Ammonia: Method of Koroleff (7); Nitrites: Griess's Method (7); Nitrates: Cadmium Reduction Method (7).

Statistical Analysis: Correlation coefficients of Bravais-Pearson were calculated on a Computec XP (Computer Technology) computer.

Results.

The highest value of ammonification activity in Cullera bay was obtained in May and the lowest in October.

In general, we have observed that the nitrification heterotrophic path is dominant over the autotrophic one. The highest value occurs in May and the nitrification activity on depth is higher than at surface.

Nitrites values were elevated during all the period of study (8.8 mg/l N/l). Nitrites and ammonia values were moderated except during the month of October where maximum values were obtained (NH₄⁺=45.6 and NO₂⁻=5.05).

No correlation have been found between microbiological activities and chemical determinations of seawater.

Discussion

The elevated value of ammonification activity found in May can be explained as result of an increase of organic matter proceeding from phytoplankton explosion and the lowest one could be due to a liberation of amine groups and the organic matter able to consume O₂ is composed of low nitrogen content compounds. Same results have been found by other authors in this area (6).

The differences found between the nitrification processes on depth and at surface can be explained by the hypothesis of photoinhibition given by Horrigan and others (8)(2) or light inhibition of nitrification. The minimum incidence of sunlight in points situated on 5 meters depth on Cullera bay produce an enhancement of nitrifying activity on depth.

No correlations have been found between microbiological activities and chemical data. This fact can be interpreted by the existence of another nitrification paths which complement the microbial activity. Similar results have been observed in others studies. (2) (4)

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