

Enumeration of anaerobic sulphite reducers from seafoods

Anaerobic sulphite reducers produce H_2S in seafoods resulting in sulphide spoilage particularly in cuttlefishes and squids. International regulatory authorities have suggested a maximum permissible level of 100/g. They are enumerated by most probable number method as well as by direct plating method.

Materials required

1. Physiological saline (0.85% NaCl)
2. Differential Reinforced Clostridial Medium (DRCM)

Procedure

The anaerobic sulphite reducers in a test sample can be enumerated by using either 5 tube or 3 tube most probable number technique (MPN), and plate count method.

I. By MPN method

MPN method is useful when samples contain very low counts (eg. less than one per milliliter). It involves inoculating appropriate volume of decimal dilutions of samples to series of tubes containing growth medium. The estimate of the counts is obtained by observing the proportion of tubes that show growth and consulting the MPN table (MacCrady's Table).

Ten gram of sample is homogenized with 90 ml of sterile normal saline and inoculated 10 ml of the homogenate (10-1 dilution) in to each of 5 tubes containing 10 ml of double strength DRCM, 1 ml each to 5 tubes of 10 ml single strength DRCM and 0.1 ml each to 5 tubes of 10 ml single strength DRCM. Anaerobic condition is created by overlying the tubes with sterile paraffin oil and incubated at 37°C in a serological water bath up to 4 days. A blackening of the media is taken as indication of a positive reaction. The number of positive tubes in each dilution inoculated is noted and the number of sulphite reducing clostridia in sample determined by consulting the MPN Table.

MPN counts can also be obtained by following the same procedure using 3 tube method and determining the counts by referring to 3 tube MPN table.

II. By Plating method

Aseptically 10 g of the given sample is weighed, homogenized with 90 ml of sterile normal saline and appropriate decimal dilutions prepared. One ml of diluted sample is transferred to sterile Petri plates, mixed with molten and cooled DRCM agar and allowed to set. Once the agar is set, an overlay of the same medium is poured and again allowed to set. The plates are incubate in inverted position in an anaerobic jar. The procedure advised by the manufacturer of the anaerobic jar to produce anaerobiosis should be followed. The plates are incubated at 37°C for 48 hours, plates observed for black colonies and counted them as sulphite reducing clostridia. In case of non-appearance of black colonies in 48 hours, incubation for another 24 hours continued.

Calculation

No. of sulphite reducing clostridia / g = (Number of colonies x Dilution)/ Weight of sample

Media composition

Differential Reinforced Clostridial Medium (DRCM)

Peptic digest of animal tissue	- 10.0 g
Beef extract	- 10.0 g
Yeast extract	- 1.5 g
Starch	- 1.0 g
Sodium acetate, hydrated	- 5.0 g
Glucose	- 1.0 g
L-Cysteine hydrochloride	- 0.5 g

Distilled water	- 1000.0 ml
pH	- 7.2±0.2

Sterilize at 121⁰C for 15 minutes. Just before use, add 0.5 ml of filter sterilized solution prepared by mixing equal volumes of 4% W/V solution of sodium sulphite and 7% W/V solution of ferric citrate to 25 ml of single strength medium or 0.4 ml and 2 ml to 10 ml and 50 ml of double strength medium respectively. Mix well.