

CHAPTER 4: WATER QUALITY ANALYSIS: ENUMERATION OF BACTERIA

Mohamad Faiz Mohd Amin and Shahrul Ismail

INTRODUCTION

Various diseases are associated with the presence of microorganisms in water. Enumeration of bacteria is the most widely used method to determine the degree of water contamination. Tests for specific organisms are usually made only when there is a reason to suspect their presence. At other times 'indicator organisms' (coliform bacteria) are used to indicate the presence of pathogens [1]. Therefore, the presence of an indicator organism suggests that contamination has occurred. Agar Plate Count or Viable Count and Most Probable Number (MPN) are commonly used to enumerate bacteria in a wastewater sample. The tests involve providing suitable bacterial breeding environments to samples and then noting the growth [1,2].

The viable count is an estimate of the number of cells. Because some organisms exist as pairs or groups and because mixing and shaking of the sample does not always separate all the cells, we actually get a count of the "colony forming units". One cell or group of cells will produce one colony and therefore be recorded as colony-forming units per mL (CFU/mL) or per gram (CFU/g) of the test material. It is almost always necessary to prepare a dilution series to ensure that we obtain a dilution containing a reasonable number of bacteria to count [1,2]. Dilutions in the range 10^{-1} (1/10) to 10^{-8} (1/100,000,000) are generally used, although with particular types of samples the range of dilutions can be restricted. For example, for water that is not turbid, the maximal dilution needed is 10^{-6} because we are aware that if there were 10^7 or more bacteria per mL, the water would be turbid.

The MPN procedure is a statistical method based on probability theory. Samples are serially diluted to the point of extinction, that is, to a point where there are no more viable microorganisms. To detect the endpoint, multiple serial dilutions are inoculated into a suitable growth medium, and the development of some recognizable characteristic, such as acid production or turbidity, is used to indicate growth (the presence of at least one viable microorganism in the diluted sample) [1,2]. The pattern of positive tests (growth) in the replicates and statistical probability tables are used to determine the concentration (most probable number) of bacteria in the original sample. Statistical MPN tables are available for replicates of 3, 5 and 10 tubes of each dilution [1,2]. The more replicate tubes used, the greater the precision of the estimate of the size of the bacterial population.

METHODOLOGY

Equipment

This is the list of equipment needed for this experiment:

- 1) Incubator (35 ± 0.5 °C).
- 2) Water Bath, Colony Counter.
- 3) Inoculated tube, pipette, 250 mL conical flask, Petri.

Chemicals

A. Culture Media

Solution A mixed with solution B, add up to 975 mL, correct to pH 7.4, add solution C and add up to 1 L.