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Microbiological enumeration of process water and slush pulp

1. Scope and significance

The following procedure is recommended primarily for the microbiological examination of process water. It is also applicable to pulp slush stock. This method is adequate for classical, heterotrophic cell counts of unencapsulated, planktonic microorganisms. This method will not give an accurate measure of the numbers of encapsulated, slime-forming cells or sessile microorganisms present. Because of the exacting technique required in microbiological procedures, reproducible results can be obtained only by a trained technician.

2. Apparatus and materials

- 2.1 *Alcohol*, isopropyl (rubbing), or ethanol (70 - 95%), for sterilizing instruments.
- 2.2 *Balance*, sensitive to 0.1 g, with a pan large enough to hold a dilution bottle.
- 2.3 *Bottles for dilution*, commercially available, pre-filled, pre-sterilized buffered dilution blanks, 99 mL, are recommended. Alternatively standard milk-dilution bottles scribed at the 99 mL level may be used. These should be filled to the 99 mL mark with deionized water and sterilized by steam sterilization as described in section 3.1.1.
- 2.4 *Sample containers*, several 125- to 250-mL wide-mouthed bottles for collecting samples. The type of bottle and cap selected should be able to withstand the sterilization procedure. Presterilized, disposable plastic bags of suitable size with flexible wire closures are satisfactory.
- 2.5 *Colony counter*, any one of several types. A hand tally for recording the count is recommended.
- 2.6 *Flaming equipment*, an alcohol lamp, Bunsen burner, or Bacti-Cinerator™ to flame tongs, forceps, and the mouths of sterile containers.
- 2.7 *Incubator*, capable of maintaining a temperature of 30 - 36°C ± 2°C.
- 2.8 *Petri dishes*, commercially available presterilized disposable (100 × 15 mm) plates, are recommended.

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2.9 *Pipets and containers*, commercially available presterilized 1.1 mL and 10 mL pipets are recommended. Automatic pipetors with sterile plastic tips or 3 mL and 10 mL commercially available sterile syringes may also be used. Containers for sampling should be sterile sample bags or sterile bottles.

2.10 *Sampling equipment*. Scoops made from tinned iron sheet. The diameter of the scoop will depend upon the opening of the sample bottle; the length is about 25.4 cm (10 in.). Wrap the scoops individually in heavy kraft paper prior to sterilization. Alternatively, large sterile spoons or dippers may be substituted for the scoops.

2.11 *Nutrient substrate*. For bacteria use, tryptone glucose extract agar, and for fungi, either potato dextrose or mycophil agar. The fungal medium should be adjusted to pH 4.0 by adding sterile 10% tartaric acid following sterilization of the agar and cooling to 45 to 50°C prior to pouring plates.

2.12 Commercially available 3M Petrifilm Aerobic count plate for bacteria and Petrifilm yeast and mold plate can also be utilized.

2.1.3 *Sterilizing equipment*, two types, suitable size: an autoclave for steam sterilization; an electrically heated hot-air oven at 165°C, with thermometer.

3. Sterilization

3.1 Depending upon the nature of the equipment to be sterilized, use one of three methods as follows:

3.1.1 *Steam heat* (autoclave). Sterilize the following in an autoclave by heating for 20 min at a minimum of 121°C, corresponding to 103 kPa (15 psi) steam pressure: (a) media (unless other conditions are specified by the manufacturer), (b) sample bottles, (c) scoops, dippers, or spoons, and (d) water blanks for dilution. If necessary, vent the autoclave for 5 min or until steam has displaced the air. Include a biological or chemical indicator strip to evaluate autoclave efficiency.

3.1.2 *Dry heat* (electrically or gas-heated oven). Sterilize the following by heating for at least 2 h at a temperature of about 165°C (329°F): (a) glass pipets and (b) forceps.

3.1.3 *Alcohol*. Immerse contact portions of forceps and spoons in alcohol. When needed for handling samples, remove, allow to drain for a few moments, then burn off the excess alcohol. Use extreme care when using an open flame around alcohol.

4. Sampling

4.1 *Frequency of sampling*. Occasional single samples are usually inadequate, since the analysis reflects only the conditions which prevailed when such samples were taken. The number and frequency of samples depend largely on the purpose of the analysis.

4.2 *Method of sampling*.

4.2.1 Use the sterile metal scoop, dipper, or spoon to collect stock samples that cannot be readily sampled in the sample stream. Dip a sample from the vat, chest, or trough, using caution to avoid contaminating the sample by the hands coming in contact with the stock, or from material adhering to the edges of the system. Transfer the sample to a sterile container and seal immediately.

4.2.2 For sampling water or dilute stock, allow the sample stream to flow directly into the sample container. If sample is obtained from a closed line, allow stock to flow one minute before sampling. Take care that the sample or container is not contaminated by the hands during the sampling.

4.2.3 Samples should be plated as soon as possible. If the sample is not plated immediately (within 30 minutes) it should be stored at 4°C. Sample should not be held for more than 24 hours. Note the time of storage in results.

5. Procedure

5.1 *Diluting tubes serial dilutions*. Choose the appropriate method below. (9 mL serial dilution tubes may be substituted for 99 mL.) If necessary, change the dilution value and labeling appropriately (1 mL sample and 9 mL dilution provides a 10^{-10}) dilution tubes.