Introduction to microbiology assignment

It enumerates the number of actual live, viable cells in the sample that form colonies on a suitable agar medium. As the optimum medium and conditions varies for one sample to another, the colony count methods provide an estimate of the number of viable cells according to the medium employed, time and temperature of incubation. Each colony that appears on the agar plate arising either from a clump of cells or from a single cell is referred as a colony forming unit (CUFF). The sample used in this experiment is active dried yeasts (DAD).

A serial dilution is performed to suspend the yeasts containing product in water so that the number of microorganisms per ml is small enough to be counted, when the sample is plated. Cells in overcrowded plates may not form colonies but may fuse which lead into erroneous measurements 1) Therefore the sample is diluted and then re-diluted successively with a known volume of diluents until it obtain appropriate colony number which is ideally between 30 and 300 colonies but more recently 25-250 range is recommended.

After that the different dilutions are plated on nutrient media using spread plate and pour plate method to allow the yeast cells to cells to grow and multiply. The number of viable cells in the sample is recorded as colonies forming units/ml or GM, COIF/ml r COIF/GM. Aerobic Mesospheric Count is one of numerous variations of colony count techniques. As the name suggests, the plates are incubated under aerobic condition and the incubation temperature is in the mesospheric range from 30 co to ICC. As the sample used is yeast, it is incubated in room temperature (ICC) for 2 days.

Literature Review A serial dilution is an accurate method of making solutions of low molar concentrations. A solution with a military greater than that which is required is accurately diluted using a suitable solvent. The dilution used in this experiment is aerial “ ten-fold” dilutions which reduces the concentration of a solution by a factor of ten that is to one-tenth the original concentration. In the preparation of serial dilution, exactly one ml of original sample is added into ml of diluents to get a 1: 10 dilution. L: 100 dilution is made by adding Mil of 1: 10 dilution to 9 ml of diluents.

The same method applies to get the subsequent dilutions until it reaches the final dilution desired. Spread plate is one common method to quantify microorganisms on solid medium. Usually 0. 1 ml of the diluted sample is dispensed on the agar plate but in this experiment 0. Ml was used instead to prevent the sample from becoming dry as the incubation period was extended. Using a sterilized device that looks like hockey stick, the sample was spread evenly on the surface of the agar and was allowed to be absorbed into the agar.

Plates were inverted and incubated. The colonies of the sample grew only on the surface of agar. Pour plate is another common method of quantifying microorganisms on solid medium. L ml of the diluted sample was dispensed into an empty, sterile Petri dish, about 15 ml of molten agar that had been cooled down in water bath to ICC was added after that. The mixture was gently swirled clockwise, anticlockwise, up and down and left and right. Having allowed the agar to set, the plates were then inverted and incubated.

Unlike the colonies in the spread plate, the colonies here formed throughout the agar, on the surface and within the medium. The advantages to spread plate technique is that the colonies termed are easily counted as they are all grow on the surface of the agar but this may bring disadvantages too due to the limited space on the plate surface and the cells with low tolerance to oxygen will not grow (4). Pour plate method eliminates such robbers because the colonies are well separated and the oxygen is sufficiently supplied to those embedded in the agar allowing them to grow and form colonies.

However, this results in difficulty in the counting as the size of the colonies varies between those on surface and in agar. Another disadvantage of pour plate method is that the weak yeast cells will be killed due to the hot molten agar causing the lesser colonies formed compared to spread plate by at least ten fold. All the plates are incubated inverted to prevent water drops forming on the top of the plate and dripping onto the plates which may cause colonies to be spread quickly exulting in unclear visibility.

Being incubated upside down, the water is absorbed back into the place as it condenses (5) Having incubated in temperature of ICC for 2 days, only plates that contain 25 to 250 colonies are chosen. The number of viable microorganisms in the original sample can be calculated by multiplying the number of colonies in the plate by the dilution factor. The units for the viable cells are COIF/ml or COIF/GM. The equation Procedure A. Serial dilution of active dry yeast (DAD) I. Log of or 1 leg of DAD was weighed and 90 ml or 99 ml of 0. % peptide water was added respectively. The yeast slurry was shaken well but gently for 10 minutes. It. To prepare a 10-2 dilution, exactly 1 ml of 10-1 was transferred into 9 ml of diluents using pipette and was

mixed well in a circular motion. The dilution technique was observed carefully. Iii. Step 2 was repeated 7 more times to obtain a dilution of 10-9. B. Plating using spread and pour plate method I. Spread plate: 0. 2 ml of 10-5, 10-6, 10-7 and 10-8 dilution was spread onto duplicate plates of malt agar using a “ hockey stick”.

The technique was observed carefully. I’. Pour plate: 1 ml of 10-6, 10-7, 10-8 and 10-9 dilution was transferred into duplicate Petri dish using a pipette and 15 ml of molten malt agar (app ICC) and was mixed gently and the agar was allowed to set. The technique was observed carefully. I The plates were incubated inverted at room temperature (ICC) tort 2 days. Lb. After incubation, plates that contain between 30-300 or 25-250 colonies were chosen; the colonies in each plate were counted and the number of yeast cells was worked out as COIF/g.