EN_83833645 Method Report

Being able to quantify microorganisms is an important part in many fields of microbiology, whether that's within research, industrial applications or healthcare. Knowing the amount of microorganisms within a sample can provide essential information about the growth of the microorganisms and the conditions of the medium in which it grows, such as pH or nutrient level. Within biological research for example, quantification can be used to determine the health of the microorganisms, or the effect of the changes to the growth conditions. Within industries such as food production, quantifying the amount of microorganism provides a way to monitor the contamination of the food product. Within healthcare, quantifying the amount of microorganisms in a sample can support a diagnostic method, for example (Brock Biology of Microorganisms, 16th edition).

There are multiple methods to quantify the viable cells in a sample, such as with digital image processing techniques with fluorescence microscopic imaging and flow cytometry (Murphy, 2005), PCR monitoring in combination with dye, and counting grown cultures, liquid or solid (Navarro, 2020). These each have their own benefits and drawbacks. Digital imaging, for example, is often very accurate, but it can end up being very expensive. Using PCR is cheap and precise, but the distinction between dead and live cells cannot be determined. Culturing the microorganisms is cheap, but it can be time-consuming and is more sensitive to human error.

Two often used methods in microbiological research are the spread plate count method and the hemocytometer in combination with methylene blue staining. The plate count method relies on the dilution of the sample in order to calculate the amount of viable cells, with the fact that only living cells will be able to form colonies when inoculated on an agar plate with the spread plate technique. The methylene blue staining can be used to determine the amount of dead and living cells in a sample, due to the fact that only dead cells will be stained by the dye. This percentage of dead versus living cells can then be applied to a hemocytometer sample of the same original sample (Bonora, 1982).

The experiment is set up in order to compare the two methods described above, which can be used to determine the amount of viable cells within a sample of baker's yeast, otherwise known as S. cerevisiae. The research question is as follows: Do the counting methods ‘’spread plate count’’ and ‘’counting chamber with methylene blue staining’’ yield the same result when used to determine the number of living cells per gram in a suspension sample of S. cerevisiae? The hypothesis states that given the amount of living cells in a counting chamber can be determined with a live-dead ratio concluded from a methylene blue stain, and only living cells would make colonies on a plate, the two methods are expected to yield the same result when used to determine the number of living cells in the sample.