EN_30453179 Review of A Research Paper

For this study, two different methods for counting Saccharomyces cerevisiae will be used. Accurately counting the number of microorganisms in a sample is an important part of current microbiological studies. It is therefore important that information about counting methods is available.

Baker's yeast has been a very important type of yeast to produce bread, beverages and biofuel in the last centuries. But in addition to that, Saccharomyces cerevisiae also has many industrial applications, because it is the best studied eukaryote. Because of this knowledge and the easy amenability to genetic manipulation, baker's yeast is an ideal model organism that is also of great importance in several biotechnological methods. (Parapouli et al., 2020)

The plate count is an easy method for counting colonies and is therefore widely used in e.g. medicinal or microbiological studies. To perform this technique, a spread plate must be made for different tenfold dilutions. It is of importance to have between 30 and 300 colonies, otherwise the colony forming units (CFU's) will give unreliable results. In the case of more than 300 colonies, some microbial cells may not form colonies, or they will fuse. But in case of too few, the result will not be accurate anymore, because the statistical significance will be too low. Colony numbers can vary with the length of incubation, incubation conditions and culture medium, which makes the cultures prone to inaccuracy. It is therefore important to eliminate these variations by keeping the variables constant. Another disadvantage is the great care that must go into preparing these plates, because they are very prone to errors such as inaccurate pipetting, inaccurate dilutions or insufficient mixing (Madigan et al.,2020).

The counting chamber is a device to help count the total cell count. The counting chamber shows a grid of squares, each with a precise volume. The number of cells per square can be counted under a microscope after which an average of the number of cells per millimeter suspension can be calculated, says Madigan et al. (2020). One big limitation is that without staining, dead cells cannot be distinguished from living ones. Therefore, to accompany this technique, a methylene blue stain is used to calculate the

live/dead ratio of yeast cells in the sample. When a cell is dead, methylene blue can easily stain the cell, but this is not the case when the cell still lives. Methylene blue is said to give consistent viability results (Trevors et al., 1983). Furthermore, erroneous counts can be made because small cells are difficult to see, and debris can be easily mistaken for microbial cells. A big advantage of using a microscopic cell count is the simplicity of this technique, which makes it easy to quickly count multiply samples (Madigan et al., 2020).

To contribute to this current knowledge, we will perform a study investigating both the plate count and counting chamber with a methylene blue stain for counting Saccharomyces cerevisiae, researching their differences and if the methods give comparable results. We hypothesize that the counting chamber and plate count will indeed give comparable results. However, we suspect that the counting chamber with a methylene blue stain is a more accurate method.