Gram and Neisser Staining is a common method used for identifying filamentous bacteria and other floc features.

To stain the sample, a series of solutions are applied to a dry sample mount for varying lengths of time. The bacteria will appear strongly positive, weakly positive, variable, or negative depending on its reaction with the dye.

Figures 1 shows an example of a gram positive filament. Gram positive bacteria is differentiated based on its ability to retain the primary dye (Crystal Violet) while Neisser positive bacteria can retain Methylene Blue. The difference between a positive and negative staining bacteria is the composition of the cell wall, which determines how the dye binds to the cell component.

Filaments that are difficult to characterise through a wet mount alone can often be identified through staining.

Each filament has a typical staining reaction, for example the majority of filaments in activated sludge are gram-negative, however some filaments such as N. Limicola and Type 0041 are typically gram positive. Unfortunately for those attempting to identify filaments, the above can also become gram-variable or gram negative, depending on their surrounding environment and growth stage. The gram stain reaction can also vary depending on the age of the sample, therefore it is important to stain the sample within 24-48 hours.

Gram positive species tend to form more robust flocs and occur in lower loaded plants. Neisser staining tests the presence of polyphosphates stored in cells, Figure 2 shows Neisser positive tetrads within a floc. Neisser staining also makes Bio-P material visible, which is the material needed for biological phosphorus removal and are observed as colonies of blue-black coloured cells.

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