

ETV methods

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CMFDA/FDA staining versus MPN method for detecting live >10-50 µm organisms in treated ballast water

NIVA has since 2006 tested BWMS according to IMO guidelines and was in 2015 approved as an independent laboratory for testing according to USCG ETV guidelines. Throughout this period NIVA has made use of both the MPN method and CFDA staining for IMO testing and in the last year the CMFDA/FDA staining method, replacing CFDA. Below we argue the case that the MPN method is the more objective scientific method for documenting that 10-50 µm organisms are rendered harmless with respect to invasiveness.

The CMFDA/FDA staining is said to measure whether an organism is killed or not by observation of an organism's ability to turn FDA or CMFDA into a fluorescent compound by an esterase enzyme which is present in most cells. Esterase enzymes within a cell will continue to do their work even if all other metabolic processes have terminated. In order to observe "kill" by the staining method cells need to be damaged in a way that either esterase enzymes are damaged or leaked out of the cells. There is large variability as to the amount of esterase within cells of different species but also within species reflecting metabolic status. Some species have a minimal amount of the esterase enzyme or have cell walls that hinder the relatively large FDA and CMFDA molecules from entering. Such cells will falsely be determined as "killed". It is up to the observer to determine whether a cell shows observable FDA or CMFDA fluorescence. Therefore the method is subjective and results are difficult to substantiate.

The MPN method estimates the density of grow able cells in a sample, giving a number with known statistical 95 % confidence limits. Regrowth of viable cells can be determined by measuring an increase in algal fluorescence in an objective and substantiated manner. The MPN method therefore is directly relevant to the purpose of the convention which is to hinder the spread of invasive species.

While disinfectant type BWMS use hypochlorite which acts primarily by disrupting outer cell membranes and subsequently releasing the esterase enzymes out of the cells, UV light type BWMS kills organism from within by damaging DNA and other macromolecules essential for sustaining metabolic activity. Grow out methods are widely accepted internationally for

BWMS approval based on the IMO guidelines. A guideline for approval of treatment systems should allow use of analytical methods that do not discriminate unduly between types of treatment systems.

Yours sincerely

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