Biological results from the ship board trials

Executive Summary

Two treatment methods were tested during the ship board trials of the MARTOB project, these were the High Temperature (HT) treatment developed by UNEW and the Deoxygenation (DEOX) method developed by SINTEF. In the HT treatment the ballast water was heated to 55-80ºC in two serial heat exchangers and then immediately cooled down again. In the DEOX treatment a nutrient solution was added to the water in the ballast tanks. The added nutrients stimulated the growth of the indigenous bacteria in the water and their growth made the water anoxic.

The sampling methodology and experimental design were influenced by the discussion at the International Maritime Organisation (IMO) regarding the ballast water management standard. This standard, adopted recently, requires that discharged ballast water shall contain less than 10 viable organisms per m^3 larger than or equal to 50µm, i.e. mainly zooplankton, and less than 10 viable organisms per ml between 10 and 50µm, i.e. mainly phytoplankton. In addition, the standard sets limits for specified indicator bacteria.

The ship based trials were carried out on the car carrier M/V Don Quijote between Egypt and Belgium (HT), and England and Panama (DEOX). A number of ballast tanks were made available for the experiments and samples were taken before, during and after treatment. A broad taxonomic analysis was carried out for zooplankton and phytoplankton. The concentration of viable bacteria was also determined. In both trials, copepods (a group of crustaceans) and nauplii (a larval form of many crustaceans) constituted 98% of the zooplankton. The phytoplankton enumeration was limited to dinoflagellates and diatoms, and the cell counts are therefore minimum values.

Both the HT and the DEOX treatment yielded water samples with a very low concentration of viable zooplankton, mostly less than 30 viable organisms per m^3 and frequently less than 10 viable organism per m^3. However, a large fraction of the zooplankton in the water samples, sometimes more than 90%, was killed during the pumping from the ballast tanks via the fire pump to the heat treatment unit and the sampling point on deck, presumably by pressure fluctuations in the fire pump. It is therefore difficult to determine how much of the mortality in the samples was due to the treatments and how much was due to the fire pump. For the HT treatment there was no significant difference between the treated water samples and the control samples with respect to copepod mortality, while the treated samples had significantly higher nauplius mortality than the controls. For the DEOX treatment there were no significant differences in the mortality for either copepods or nauplii between the samples from the treated tanks and the controls.

For the HT treatment, or more correctly the HT + fire pump treatment, increasing the treatment temperature above 60ºC did not improve the effectiveness. There was an increase in nauplius mortality over time, i.e. the longer they had been in the tank the more effective the treatment.

Due to the killing effect of the fire pump, it is difficult to quantify the effect of the HT treatment. However, the concentration of survivors in the HT treated samples was only around 10% of the concentration in the control samples. The organisms in the control samples had only been subjected to the fire pump, while the HT treated samples had been subjected to both the fire pump and the HT treatment. This indicates that the HT treatment killed around 90% of the organisms that survived the

fire pump. The zooplankton that survived the fire pump are likely to be among the hardiest, and the result therefore indicates that the HT treatment alone would have killed at least 90% of the zooplankton in the ballast water, and probably considerably more. However, even in combination with the fire pump the HT treatment achieved the new IMO standard of less than 10 viable organisms per m^3 only in 40% of the trials.

Both in the control tanks and the treated tanks in the DEOX trial, the total concentration of zooplankton, i.e. both live and dead, decreased during the trial. The decrease was fastest in the treated tanks and after 5 and 7 days the average total concentration was significantly lower in treated than in untreated water; 25-50 org/m^3 in treated versus 400-430 org/m^3 in untreated water. The average concentration of *viable* organisms in the water samples from the treated tanks was only 1-3 per m^3, while the samples from the untreated tanks contained 10-150 viable organisms/m^3. The DEOX treatment in combination with the killing effect of the fire pump therefore achieved the new IMO standard, but it is not possible to determine if this standard would have been achieved without the fire pump.

Both in the HT and the DEOX trial the concentration of phytoplankton (dinoflagellates + diatoms) was very low from the start, around 1 cell per ml. No significant effect of the HT treatment was observed, but as the chlorophyll level in the HT samples was too low to yield meaningful results and the viability of the phytoplankton cells was not determined, no conclusion can be made as to the effect of the treatment. Also for the DEOX treatment, the lack of viability assessment makes the results difficult to interpret, but it would seem unlikely that deoxygenation is an effective method of reducing diatoms and dinoflagellates. All the phytoplankton cell counts in the treated samples, both from the HT and the DEOX trial, were below the IMO standard, but the phytoplankton concentration was below this standard from the start in both trials. This highlights the fact that several ship based trials would be required to ensure that the treatments were tested over a range of concentrations of plankton.

In the HT trials the concentration of viable bacteria in the water samples was reduced with around 95% for all the temperatures tested (55-80ºC). The indicator bacteria in the IMO standard (*V. cholerae*, *E. coli* and intestinal enterococci) were not analysed in these experiments, but the results indicate that, if present, the HT treatment would have reduced the viability of *V. cholerae* and *E. coli* with at least 95% and possibly considerably more. Whether or not this is enough to achieve the new IMO standards for the indicator species depend upon their concentration before treatment, but in most situations it is likely that a reduction with two orders of magnitude will be enough. Because some intestinal enterococci are relatively heat resistant, the effect of the HT treatment on this group needs to be tested in practical experiments.

In the DEOX treatment the concentration of viable bacteria increases during the process. The concentrations of the indicator species, if present, are not likely to increase, but their fate during the DEOX treatment was not studied and is still unknown.

The results of the trials highlighted some of the problems associated with sampling and analysing ballast water on board vessels. In particular, quick and reliable methods for counting and assessing the viability of different groups of organisms require much more development. Furthermore, a large variation was observed in the number of organisms in successive samples taken from the same ballast tank, and this shows that the distribution of plankton within the ballast tanks is not homogeneous. This has important implications for how sampling should be performed to test for compliance with the new IMO standard.