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# Final report of the land-based and shipboard testing of the SEDNA®-system

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OZ Royal Netherlands Institute for Sea Research

# Final report of the land-based testing of the SEDNA®-System, for Type Approval according to Regulation-D2 and the relevant IMO Guideline

**(April – July 2007)**

# Final report of the shipboard testing of the SEDNA®-System, for Type Approval according to Regulation D-2 and the relevant IMO Guideline

**(June – December 2007)**


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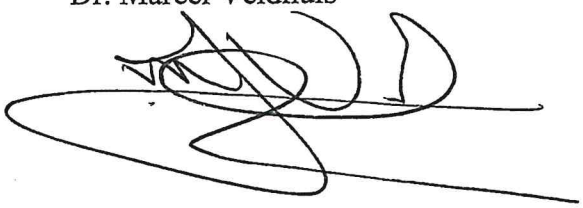
**(April – July 2007)**

Dr. Marcel Veldhuis



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# Content

## Executive summary

The SEDNA®-System, a ballast water treatment system, was tested according to Regulation-D2 (D2-Standard), the IMO Guidelines for Type Approval testing (G8) and for approval of BWT systems that make use of active substances (G9) in the spring and early summer of 2007 in the harbour of the Netherlands Institute for Sea Research. Tests were successful and the BWT system performed on average much better than Regulation-D2 by achieving values for organisms well below the requirements of the D2 Standard. This system should therefore be regarded as an effective and safe way of cleaning ballast water in ships, thereby minimizing the risk of new invasions originating from the ships ballast water.
Sediment was removed by the hydrocyclone and filters and organisms were killed by the active substance PERACLEAN® Ocean. In general the organisms disintegrated completely and the remaining debris was found as an amorphous structure or as dissolved organic carbon. Upon discharge no residual effects of the chemical treatment to the receiving environment could be noted. The active substance was decomposed usually within 24 h and the bacteriostatic agent ($H_2O_2$) was degraded within 3 days after addition. The by-product, acetic acid, reduced the pH of the water temporarily but was effectively and completely mineralized and respired by heterotrophic bacteria. Although this caused a temporary reduction in the ambient oxygen concentration no anoxia was observed. Upon discharge after 5 days only in the pure treated water and the lowest dilution (1:1) with ambient seawater tests showed a slight, but insignificant, effect of remaining acetic acid.

## Zusammenfassung

Das SEDNA®-System zur Behandlung von Ballastwasser wurde im Frühjahr und Sommer 2007 gemäß Regulation-D2 (D2-Standard), sowie der IMO Richtlinien zu Tests für eine Typzulassung (G8) und denen für eine Zulassung von Systemen, welche aktive Substanzen verwenden (G9) im Hafen des Königlich Niederländischen Meeresforschungsinstitut (NIOZ) getestet. Die Tests waren erfolgreich und das System zeigte eine bessere Leistung, als gefordert. Die Organismenanzahlen nach der Behandlung lagen deutlich unter den Anforderungen des D2-Standards. Das System sollte demzufolge als effektive und sichere Möglichkeit zur Behandlung von Ballastwasser betrachtet werden, die dazu beitragen kann, weitere biologische Invasionen zu verhindern.
Sedimente wurden durch die Hydrozyklone und den Filter entfernt. Organismen wurden durch die aktive Substanz PERACLEAN® Ocean abgetötet. In der Regel wurden die Organismen vollständig aufgelöst und die Überreste konnten nur als amorphe Strukturen oder in Form von gelöstem, organischem Kohlenstoff nachgewiesen werden. Beim Ablassen des behandelten Wassers wurden keine Residualeffekte der chemischen Behandlung auf die Umwelt festgestellt. Die aktive Substanz wurde normalerweise binnen 24 h zersetzt und die bakteriostatische Komponente ($H_2O_2$) wurde innerhalb von 3 Tagen nach der Dossierung abgebaut. Das Nebenprodukt des Abbaus, Essigsäure; senkte den pH-Wert des Wasser zeitweise, wurde aber effektiv und vollständig von heterotrophen Bakterien zersetzt. Obwohl dadurch eine temporäre Abnahme der Sauerstoffkonzentration ausgelöst wurde, wurden keine anoxischen Bedingungen festgestellt. Geringfügige Effekte der Essigsäure konnten experimentell nach den 5 Tagen Aufbewahrungszeit nur in unverdünntem, behandeltem und 1:1 mit Seewasser verdünntem, behandeltem Wasser nachgewiesen werden.

## Acknowledgements

The authors thank the technical staff of the NIOZ and in particular Anna Noordeloos, Swier Oosterhuis, Robert Lakeman, Santiago Gonzalez for making these tests possible. Without their help and devotion the report would never have the standard it has now. Special tanks also to Yolanda van Iperen for providing the species list of plankton in the Wadden Sea and Lothar Reinecke, Matthias Welle and Andreas Meinhardt for running the SEDNA® system without any technical problem and Bernd Hopf and Michael Volkmer for assisting us with the PERACLEAN® Ocean measurements. We also would like to thank the commander and the unit of the Royal Dutch Corp of Marines at Texel for their assistance.

## 1. Introduction

Ships transport 5-10 billion tons of ballast water annually all over the globe (Endresen et al. 2004). The ballast water is loaded with particulate sediment and an enormous variety of (living) organisms, which ranges from juvenile stages, larvae and eggs of fish and larger zooplankton (Williams et al. 1988; Carlton and Geller 1993) to macroalgae, phytoplankton (Hamer et al. 2000; Hallegraeff et al. 1997), bacteria and viruses (Gollasch et al. 1998). In general these organisms belong to the natural ecosystem in and around the port of origin but they might not be occurring naturally in the coastal waters and port of destination at the end of a ship's journey. In hundreds of cases around the world, this has resulted in severe damage to the receiving ecosystem and to human health, because these non native organisms developed into a plague. This often has a high impact on the ecosystem and can cause economical damage (Hoagland et al. 2002), as it results in a decrease of stocks of commercially valuable fish and shellfish species and occasionally outbreaks of diseases such as cholera (Ruiz et al. 2000; Drake et al. 2001). If action is not taken the problem of invasive species will increase in an exponential manner for several reasons. Ships are getting larger, faster and the amount of traffic across the oceans is expected to increase rapidly during the coming decades, and therefore also the change of non-indigenous organisms to have large enough number for settling and expanding. Our effort to reduce pollution of ports and coastal waters also improves the quality of the aquatic environment in these areas and therefore increases the susceptibility to invasive organisms. Originally not intentionally meant but organisms in ballast water will experience favourable conditions for settling and growing. The problem of invasive species is considered as one of the 4 major threats of the world's oceans next to land-based marine pollution, overexploitation of living marine resources, and physical alteration/destruction of habitats

To minimize these risks for the future, the International Maritime Organization (IMO) of the United Nations has adopted the Ballast Water Convention in 2004 (Anonymous 2005). The Convention states that finally ALL ships (>50,000 in number) should install proper ballast water treatment (BWT) equipment on board between 2009 and 2016. As a temporary and intermediate solution for the time being ships may reduce the risk of invasive species by performing ballast water exchange during their voyage when passing deep water (>200 m depth and 200 NM from the coast). Ballast water exchange faces many problems as to feasibility, safety and efficacy For a large part of ships' voyages the required depth and/or distance to shore requirements are never met; BW exchange can affect the ships construction stability and in rough seas exchange is not possible because of the risk to ship and crew. Treatment of ballast water is therefore considered to be the best solution of reducing the risk of invasive species.

During the recent years numerous solutions for treatment of ballast water have been mentioned and tested with the ultimate goal to reduce the amount of organisms in ballast water (Rigby and Taylor 2001). However, next to a high efficacy there is more needed for a BWT system to be good system. Next to biologically effective the system should be practicable, environmentally acceptable and also cost effective.
Despite the fact that the treatment technology for drinking-, waste- and process water is well-developed none of these techniques is directly applicable to ballast water (Rigby and Taylor 2001; MEPC 49/2/13, 2003). Besides reducing the load of organisms the sediment load should be reduced as well. There are also considerable differences in ships operation, types of ships and the amount of space available for a ballast water treatment system on board and the way ships are operated. Ballast water treatment will develop into a new field of technology of its own with a commercial market estimated for the next 10 years in the order of 8 billion Euro (Haskoning 2001).

As a primarily scientific research institute NIOZ is defining its role in the certification process as to study
1) the abundance and biodiversity of organisms prior, during and after a treatment with the SEDNA®-System (efficacy of the BWT system),

2) to determine the viability status of the remaining organisms during discharge,
3) to determine the environmental performance of the active substance and/or its degradation products after discharge (environmental impact).

This research strategy allows for more in depth testing, while it includes ALL organisms and not only the size classes as specified in the Convention D2 standard.

## Description of the treatment facility

**NIOZ Royal Netherlands Institute for Sea Research**

NIOZ Royal Netherlands Institute for Sea Research is the National Oceanographic Institute of the Netherlands. NIOZ is part of the Netherlands Organization for Scientific Research (NWO). The institute employs around 200 people and the annual budget is approximately 20 million.
The mission of NIOZ is to gain and communicate scientific knowledge on seas and oceans for the understanding and sustainability of our planet. The institute also facilitates and supports marine research and education in the Netherlands and in Europe.

In order to fulfil its mission, the institute performs tasks in three specific fields.
Research: The emphasis is on innovative and independent fundamental research in continental seas and open oceans. The institute also carries out research based on societal questions when this merges well with its fundamental work. The senior scientists at NIOZ all participate in international research projects.
Education: The institute educates PhD and other students of universities and schools for professional education. Together with universities NIOZ also organises courses for PhD students and master students in the marine sciences. A number of our senior scientists of NIOZ is also appointed as professor at Dutch and foreign universities.
Facilitary services: NIOZ invites marine scientists from Dutch and foreign institutes and universities to write scientific proposals involving the institute's research vessels, laboratories, and the large research equipment, which is often designed and built by the institute's own technical department.
The basic oceanographic disciplines studied at NIOZ are physics, chemistry, biology and geology. Multidisciplinary research is regarded as one of the main strengths of NIOZ.

More information on www.nioz.nl

**Portrait of HAMANN AG (producer of the SEDNA®-system)**

HAMANN AG has been founded in 1972 by Mr. Knud Hamann under the name of Hamann Wassertechnik GmbH. Concentrating on physio-chemical waste water treatment plants for all kinds of ships, the company constantly grew in both, turnover and staff. Over the years the product range of Hamann Wassertechnik increased continuously. As a consequence, different branches were established for the handling of spare parts, for emission control and for ballast water treatment. All of these branches were joint together under the umbrella of HAMANN AG in 2004, with the addition of freshwater production and in-house workshop facilities completing the picture.
HAMANN AG is certified according to ISO 9001, which guarantees the upmost possible quality and reliability in the products and the workmanship. With 70 people working at the head office in Hollenstedt and with a constantly growing number of agents and service stations around the globe (currently, 31 agents are under contract), HAMANN AG offers custom made products as well as a complete after sales service.

More information on: www.hamannag.com

**Ballast water treatment - The SEDNA® -System**
The SEDNA®-System operates in-line during the uptake of the ballast water. It is based on a modular concept which includes a two-step physical separation (hydrocyclone and self-cleaning filter) as well as a secondary treatment with an oxidising agent (PERACLEAN® Ocean). The system is able to handle ballast water pump capacities from 250m³/h up to 1000 m³/h in a single module. A combination of modules is able to serve larger pump capacities. For Navy vessels and Mega Yachts smaller plants are in the engineering phase. Various configurations are feasible but the following are the three basic designs:
- Containerised system
- Skid mounted system
- Modular system

A full scale plant is in continuous operation aboard a newly built container ship since mid 2006.

**Portrait of Evonik Degussa GmbH (producer of PERACLEAN® Ocean)**
Evonik is a newly formed international industrial group with a workforce of about 43,000 and activities in more than 100 countries all over the world. The business areas are Chemicals, Energy and Real Estate. The operations are bundled within 14 business units which report directly to the Group board of management. The Corporate Center in Essen, Germany, performs the Group's strategic management functions. The business units and Corporate Center are supported by Shared Services which provide cross-locational services such as procurement and payroll accounting.

Evonik Degussa GmbH is the producer of PERACLEAN® Ocean, a chlorine-free disinfectant especially designed for treating ballast water. The product contains as active substance peracetic acid (PAA, 15 %) and $H_2O_2$ (14.3 %, effectively preventing re-growth). During the reaction PAA is converted into acetic acid (HAc), which is an organic carbon source normally also present in natural aquatic environments.
PERACLEAN® Ocean has been granted Basic Approval by IMO's Marine Environment Protection Committee on the 24th of March 2006, additional testing according to the appropriate IMO Guidelines has been carried out in order to provide comprehensive information needed to apply for Final Approval (G9 documentation).
More information on: www.evonik.com

## 2. The test facility

The land-based tests were carried out at the Royal Netherlands Institute for Sea Research (NIOZ), Landsdiep 4, 1797 SZ 't Horntje, Texel, the Netherlands, from March till July 2007 (www.nioz.nl).

The NIOZ test site is equipped with 3 coated concrete tanks of 300 m³ volume each to simulate the ballast water tanks of the ship (Figure 1). The tanks were (steam)cleaned after each test run. Water samples can be taken from bypasses of the standard piping used to fill and to empty the tanks or directly from the tank at outflow at ca. 1 m from the bottom.

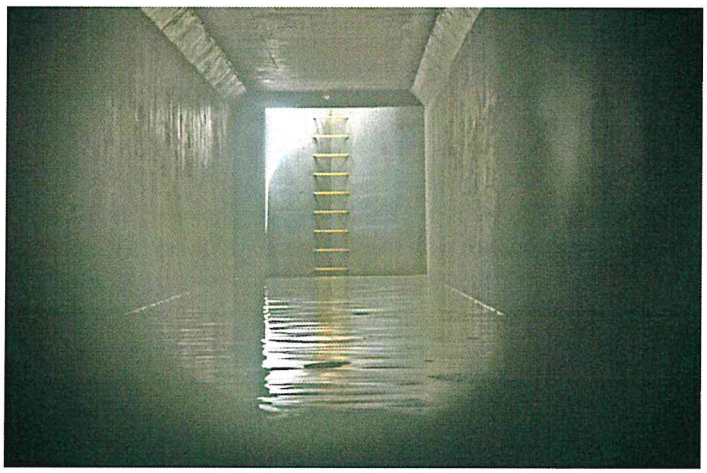

**Figure 1:**
Inside view of one of three subterranean water tanks

According to the requirements of the Guidelines G8, sampling points are fitted before the treatment system and directly after the system. Samples varying in volume from 500 mL up to 1 $m^3$ were taken using clean sampling containers. Sampling containers and all further handling of the samples were separated in a control and a treated set to avoid cross contamination by the active substance. The basic handling, such as concentrating, filtration and chemical analysis was done at the test site. Different samples (1 to 10 L) were transported to the institutes laboratories for further special analysis. For re-growth experiments 10 L of sample was transported (Nalgene bottle) to a climate room for incubation experiments (ca. 12 – 15 °C; a light:dark regime of 16:8 h and 100 µmol quanta. $m^{-2}.^{-1}$)
The SEDNA®-system is connected to a typical ballast water pump which was located in the NIOZ harbour. This is a pristine harbour with direct access to the Wadden Sea and the origin of the test water changes with the tide. Furthermore, provision were made to allow the addition of salt water and / or freshwater in order to adjust the salinity of the natural water of the NIOZ harbour to the required test conditions of brackish water and marine water with a minimum of 10 PSU difference. A detailed description of the test installation is presented in figure 2.

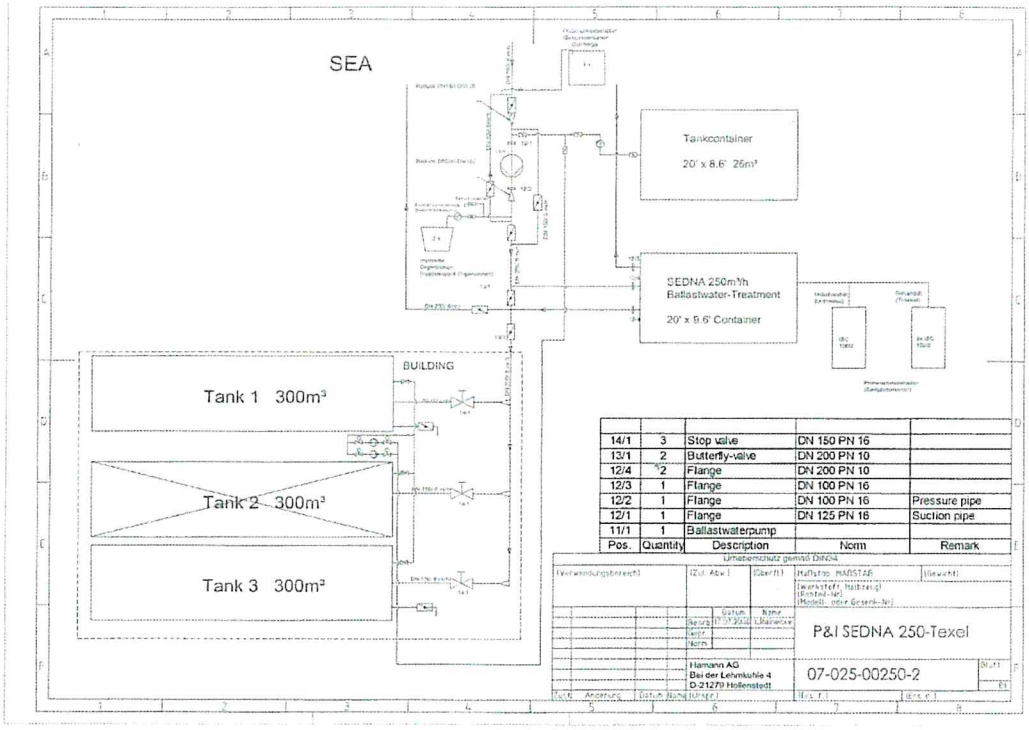


**Figure 2** : P&I diagram of SEDNA® installation at NIOZ with points of sampling

## Technical description of the SEDNA system

The SEDNA®-System operates in-line during the uptake of the ballast water. It is based on a modular concept which includes a two-step physical separation as well as a secondary treatment with an oxidizing agent.
The 1st step of the physical separation is a newly developed hydrocyclone that was specially designed for ballast water applications. The number of hydrocyclones needed depends on the flow rate of the ballast water pump.
The 2nd step of the physical separation is a compact, self-cleaning filter with 50µm mesh arranged in separate elements. The back-flushing of the filter is triggered by the differential pressure (max. 100 mbar), and the filter elements are cleaned one by one with seawater without addition of any cleaning substances. Any concentrates from the hydrocyclones (mainly sediments) and any rejects from the fine filter remain at their point of origin and no new waste lines are produced when the system is operated on a ship.
The SEDNA®-system is self-monitoring (PLC controlled) with an automated bypass according to the class requirements of the Germanischer Lloyd (GL). All materials and specifications are selected in order to meet the class requirements of the Germanischer Lloyd for ballast water systems and electric installations.
The SEDNA®-system used during the tests at NIOZ has been built to the same standards as the double 250 m³/h containerised SEDNA®-system that is also installed on the test ship (certified by Germanischer Lloyd).

The secondary treatment is the chlorine-free disinfection with PERACLEAN® Ocean (150 ppm = 150 mg/L), which is dosed automatically to the ballast water after the physical treatment. PERACLEAN® Ocean has been granted Basic Approval from the MEPC at the 54th meeting in March 2006 (MEPC 54/21). This compound contains as active substance PerAcetic Acid (PAA, 15%) and $H_2O_2$ (14.3%). Inert ingredients are water (43.5 %), acetic acid (26.5 %) and a stabilizer (<1 %). At the time of injecting the product into the ballast water the concentrations are 22.5 mg/L PAA and 21.5 mg/L $H_2O_2$. Afterwards, peracetic acid is converted into acetic acid and together with the acetic acid already present in the original formulation this results in a final acetic acid concentration in the treated ballast water of >50 mg/L. Acetic acid is easily biodegradable. Hydrogen peroxide is completely degraded resulting in water and oxygen.

During a pilot study at the NIOZ harbour in 2004, no well-described test methods were available. Thus we had to develop these methods from the requirements laid-out in Guidelines for uniform interpretation of the IMO ballast water convention and the methods developed for our fundamental scientific research on plankton. In practice sampling devices and sample volumes had to be adapted taking into account the extremely low thresholds of maximum allowed numbers of organisms per volume. For the large organisms (>50 µm) a new type of sampling net was designed by Hydrobios (Kiel, Germany) to concentrate the organisms from several $m^3$ of ballast water

after it had been in the tanks. For the small organisms (10 - 50 µm) the required sample sizes fitted well with the volumes used for semi automated analysis by flow cytometry (Dubelaar et al. 1999; Veldhuis and Kraay 2000). In addition to size class and maximum number of organisms per unit volume, the IMO D2-Standard refers to the presence of viable organisms rather than total organisms. To differentiate between life and dead plankton, a newly developed staining method using the dye SYTOX Green was successfully applied (Brussaard et al. 2001; Veldhuis et al. 2001; Casotti et al. 2005). This method is based on the integrity of the cell membrane which is intact in life cells but is disrupted when cells are exposed to stress, e.g. caused by physical damage or chemical treatment.

The physical components of the tested BWT system (hydrocyclone and filters) turned out to be effective in reducing the numerical abundance of the larger organisms but the chemical treatment was still necessary to reduce the number of living organisms in all size classes to below the maximum acceptable number. Moreover, the bacteriostatic compound of the chemical ($H_2O_2$) delayed the regrowth of total bacteria by approximately 10 days.

Currently our test protocols are communicated with various national and international agencies. Upon agreement by national administrations and in collaboration with specialised classification societies for ships, it is our goal to develop these protocols further to official Guidelines used for Certification together with our international partners in Europe, the United States and Asia.

NIOZ has the ambition to become the European test centre for the certification of BWT equipment and related research. To achieve this, we have to develop or adapt automated analysing methods for phytoplankton, zooplankton and bacteria. We have had the opportunity to collaborate with the German firm Hamann AG to optimise our test methodologies and at the same time work on the certification of their SEDNA®-system. The NIOZ harbour was used as the land-based testing site for brackish water and high saline water under regulation G8 of the IMO ballast water convention and testing was done according to a test protocol approved by the Federal Maritime and Hydrographic Agency of Germany (BSH).

## 3. Requirements to meet the Regulation-D2

According to the D2-Standard of the IMO/MEPC Convention of 2004 (Anonymous 2005) ships that meet the requirements of the Convention by meeting the ballast water performance standard must discharge:

1) Less than 10 viable organisms per cubic metre greater than or equal to 50 micrometers in minimum dimension;
2) Less than 10 viable organisms less than 50 micrometers in maximum dimension and greater than or equal to 10 micrometers in minimum dimension and
3) Less than the following concentrations of indicator microbes, as a human health standard:

1. Toxicogenic *Vibrio chlorerae* (serotypes O1 and O139) with less than 1 colony forming unit (cfu) per 100 milliliters or less than 1 cfu per 1 gramme (wet weight) of zooplankton samples:
2. *Escherichia coli* less than 250 cfu 100 milliliters;
3. intestinal Enterococci less than 100 cfu per 100 milliliters.

The present D2-Standard is defined as a standard for the water characteristics at discharge. Furthermore, with exception of some indicator microbes (point 3) organisms < 10 µm are completely excluded. This is certainly an omission since this size class in particular includes numerous phytoplankton species characterised as Harmful Algal Blooms (HABs).
Nevertheless, the standard is clear with respect to the maximum number of organisms remaining. On the other hand a proper definition of the dimensions of organisms is still subject of (academic) discussion. Moreover, as an operational definition for viable organisms the IMO is using: organisms and any life stages thereof that are living, but a more adequate (scientific) definition is: an organism that is able to complete it's life-cycle, including reproduction (DNA replication).

In addition to the requirements for the D2-Standard we have adopted a variety of methods and techniques to determine abundance, sizes and the viability status of different types of organisms. Moreover, we extended our research effort to examine not only the fate of the organisms in the large-scale ballast water basins but also took subsamples for incubation under optimal growth conditions to study the growth potential of remaining organisms or survival stages such as eggs, cysts or dormant cells over a longer period than the recommended 5 days.

## 4. Requirements to meet: guideline G8

Next to the D2-Standard two guidelines were developed by the IMO as a framework for approval of ballast water treatment systems (G8) and approval of the use of active substances in ballast water treatment systems (G9). For land-based testing MEPC 53/Annex 3 (Anonymous 2005) was compiled of which the most relevant parts will be presented below. These guidelines were generically designed to meet the conditions of a broad range of potentially effective treatment techniques to be tested in typical port and environmental conditions found across the globe. Most test protocols therefore require extensions of the test design to cover the specific aspects of the treatment. The land-based testing serves to determine the biological efficacy of the BWT systems under consideration for Type Approval under more or less controlled and replicable conditions. This is intended to ensure that the efficacy of the equipment is consistent and can be shown repeatedly. The test set-up should therefore be representative of the characteristics of the arrangements used and the type of environment the BWT system was designed for.

One of the main criteria in the G8 test requirements is the salinity range and related to this the differences in Total Suspended Solids (TSS), Particulate Organic Carbon (POC) and Dissolved Organic Carbon (DOC). This resulted in three main categories of test conditions (Table 1).

**Table 1:** Three different salinity ranges and minimum concentrations of TSS, POC and DOC in the water.

| Salinity | | | | |
|---|---|---|---|---|
| Parameter | > 32 PSU | 3 – 32 PSU | < 3 PSU | units |
| Total Suspended Solids | > 1 | > 50 | > 50 | mg/L |
| Particulate Organic Carbon | > 1 | > 5 | > 5 | mg/L |
| Dissolved Organic Carbon | > 1 | > 5 | > 5 | mg/L |

Additional experiments and previous tests (Veldhuis et al. 2006) showed that the SEDNA®-system was not affected by differences in salinity, sediment or organism load. The physical steps, hydrocyclone and filtration, are unaffected by salinity but also the reactivity of the active compound (PERACLEAN® Ocean) is not altered in the presence of high concentrations of salt. It was for this reason that the Type Approval tests were conducted at the intermediate (3 – 32 PSU) and high salinity (>32 PSU) regions. Moreover, the only difference in composition of the test water between the freshwater and intermediate salinity water is the presence or absence of (sea)salt. All other minimum requirements for TSS, POC and DOC for these two water types are identical (Table 1).
A further requirement is that the difference between the two salinity regimes should be at least 10 PSU. The test water, originating from the Wadden Sea, and the actual sampling did vary with the tide and as a result salinity was subject to variations. To assure the 10 PSU salinity difference it was decided to have the possibility of adding fresh surface water (this water was freshly taken from Lake IJssel, a freshwater basin with an outflow into the Wadden Sea and close to the institute) and upgrade Wadden Sea water by enhancing the salinity (brine solution of commercially available sea salt; ca. 18%). As target number the freshwater addition was adjusted to a salinity of ca. 23 PSU for the low salinity regime and ca. 33 for the high salinity regime. In practice ca. 15 % (v/v) of freshwater was added during the low salinity tests and about 4% of brine solution (Instant Ocean®), during the high salinity test runs. In order to compensate the dilution of the TSS by the freshwater some extra sediment (taking from a nearby mudflat) was added as well. These additions were made close at the pump site, to ensure proper mixing, with a constant flow rate and done during filling of the control and the treated ballast tank.

**Biology**

The guideline G8 also defines criteria for the number of diversity of the organisms to be met during Type Approval testing (Table 2). These criteria should be met for all three salinity regions.

**Table 2:** Minimal numbers and species diversity required at intake for different size classes and groups of organisms.

| Influent test water | | |
|---|---|---|
| Parameter | units | Remarks |
| organisms ≤ 50 micron | > $10^5$/ $m^3$ | At least 5 species from at least 3 different phyla/divisions |
| 10 ≥ organism size ≤ 50 micron | > $10^3$/ mL | At least 5 species from at least 3 different phyla/divisions |
| heterotrophic bacteria | > $10^4$/ mL | Not further defined |

The test water should contain minimum densities of plankton which are typical densities encountered in the Wadden Sea during the annual spring bloom in April/May. With respect to the species diversity, the Wadden Sea is known for its natural richness in organisms and during the test period (April – July) indeed a large diversity in organisms, adults, juveniles, eggs, etc. was encountered.
An important aspect, so far not recognised in the guidelines (G8), when dealing only with natural populations of organisms in the influent of the test water is the natural seasonality of species and blooms. The actual onset of the spring bloom is characterised by a dominance in phytoplankton, but usually lacks high zooplankton abundance. Only at a later stage zooplankton starts to increase in abundance, subsequently due to predation it will diminish the numerical abundance of (smaller sized) phytoplankton component.
Furthermore, for the high salinity range, the composition of the organisms in the water resembles that of a typical oceanic environment. This implies an increase of smaller sized cells, down to the micrometer scale, and also a dramatic decline in the number of larger (>10 micron) organisms. So far this shift in community structure has not been accounted for when using natural plankton for testing.

**Human pathogens**

**Table 3:** Maximum allowed numbers of 3 indicator groups of indicator microbes in the effluent test water on discharge. cfu: colony forming units

| Effluent test water | | |
|---|---|---|
| Parameter | | Remarks |
| Toxicogenic *Vibrio chlorerae* | < 1 cfu/100 mL or< 1 cfu/ g wet weight of zooplankton | serotypes O1 and O139 |
| *Escherichia coli* | <250 cfu/ 100 mL | |
| intestinal Enterococci | <100 cfu/ 100 mL | |

Within the group of prokaryotic microbes only bacteria and more specifically the heterotrophic group (Table 2) has been defined by the standard but for completeness this should include all bacteria and presently also Archeae. While these microbes are part of the natural community in the aquatic environment the indicator microbes (Table 3), i.e. the human pathogens, are introduced as part of human activity and often associated with sewage discharge. In the present research all microbes have been included as a bulk parameter, the number of heterotrophs as a viable component as well as the viability of the whole microbial community has been determined.

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dfds2) to determine the viability status of the remaining organisms during discharge,
3) to determine the environmental performance of the active substance and/or its degradation products after discharge (environmental impact).

This research strategy allows for more in depth testing, while it includes ALL organisms and not only the size classes as specified in the Convention D2 standard.

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Research: The emphasis is on innovative and independent fundamental research in continental seas and open oceans. The institute also carries out research based on societal questions when this merges well with its fundamental work. The senior scientists at NIOZ all participate in international research projects.
Education: The institute educates PhD and other students of universities and schools for professional education. Together with universities NIOZ also organises courses for PhD students and master students in the marine sciences. A number of our senior scientists of NIOZ is also appointed as professor at Dutch and foreign universities.
Facilitary services: NIOZ invites marine scientists from Dutch and foreign institutes and universities to write scientific proposals involving the institute's research vessels, laboratories, and the large research equipment, which is often designed and built by the institute's own technical department.
The basic oceanographic disciplines studied at NIOZ are physics, chemistry, biology and geology. Multidisciplinary research is regarded as one of the main strengths of NIOZ.

More information on www.nioz.nl

**Portrait of HAMANN AG (producer of the SEDNA®-system)**

HAMANN AG has been founded in 1972 by Mr. Knud Hamann under the name of Hamann Wassertechnik GmbH. Concentrating on physio-chemical waste water treatment plants for all kinds of ships, the company constantly grew in both, turnover and staff. Over the years the product range of Hamann Wassertechnik increased continuously. As a consequence, different branches were established for the handling of spare parts, for emission control and for ballast water treatment. All of these branches were joint together under the umbrella of HAMANN AG in 2004, with the addition of freshwater production and in-house workshop facilities completing the picture. HAMANN AG is certified according to ISO 9001, which guarantees the upmost possible quality and reliability in the products and the workmanship. With 70 people working at the head office in Hollenstedt and with a constantly growing number of agents and service stations around the globe (currently, 31 agents are under contract), HAMANN AG offers custom made products as well as a complete after sales service.

More information on: www.hamannag.com

**Ballast water treatment - The SEDNA® -System**
The SEDNA®-System operates in-line during the uptake of the ballast water. It is based on a modular concept which includes a two-step physical separation (hydrocyclone and self-cleaning filter) as well as a secondary treatment with an oxidising agent (PERACLEAN® Ocean). The system is able to handle ballast water pump capacities from 250m³/h up to 1000 m³/h in a single module. A combination of

Within the whole microbial community the number of heterotrophic bacteria was determined as well as *E. coli* and total enterococci. The test area of the institute is part of a tidal estuary of the Wadden Sea, which is essentially a pristine environment. Moreover, waste water treatment is highly developed in the Netherlands. Therefore, numbers of these human pathogens during the tests were expected to be low for most of the sampling period. On the other hand the end product of the active substance is acetic acid, which is a typical (natural) substrate found in the aquatic environment that is known to be consumed by bacteria. The present quantities of acetic acid are high enough to boost the activity of in particular heterotrophic bacteria. Therefore special attention was given to the degradation rate of the elevated acetic acid concentration and its effect on the bacteria community.

## 5. Test procedures

A variety of methods was applied to examine the biological efficacy of the SEDNA®-system for the different categories of organisms during the two test series. For detailed description we refer to read the outline of the official test protocols for the SEDNA®-system (Anonymous 2007). Sample handling and volumes were according to the description of the guideline for BWT testing (G8) or described in detail when these guidelines were insufficient or other consideration were taken into account. Subsamples were taken at random and when needed from multiple bottles or containers. As indicated previously there was great emphasis on analysing of the freshly taken samples and having multiple methods to examine numerical abundance and viability. Besides various biological samples there was also a basic set of physical and chemical parameters which were monitored prior, during and after discharge. A brief description of each parameter and how it has been analysed is given below.

**Physical and chemical properties of test water**

**Temperature**

The water temperature was measured using a calibrated thermometer.

**pH**

The pH-level was measured using a calibrated pH-meter.

**Salinity**

For salinity ca. 250 - 500 mL water was sampled and stored at room temperature (glass bottles) until analysis by direct measurement in the laboratory at NIOZ. Salinity of the water was measured after each test cycle using a refractometer (Atago) calibrated against 0 and 33 PSU standard (sea)water. The accuracy of the salinity measurement was 0.5 PSU.

**Dissolved Oxygen**

The spectrophotometric method of the Winkler method (Winkler, 1888, Pai et al. 1993, Reinthaler 2006) was used to determine the oxygen concentration in the water. Samples were taken using gastight tubing which was specially fitted to the sampling tubing that was used to sample the ballast simulating tanks. The coded glass bottles are flushed at least three times their volume (ca. 120 mL) with water.
The sample bottles were stored in a dark container filled with water of the same temperature as the samples until further analysis at the laboratory. In the laboratory 1 mL $H_2SO_4$ is added prior to measuring the OD at 456 nm with a Hitachi U-3010 Spectrophotometer. Oxygen concentration is calculated using standards and expressed as µM $O_2$/L (or mg/L $O_2$ = µM $O_2$ * 0.032)

**DOC**

The concentration of dissolved organic carbon (DOC) was measured according to Reinthaler & Herndl (Reinthaler and Herndl 2005). Samples for DOC (15 mL) are filtered through GF/C filters and sealed in pre-combusted glass ampoules after adding 50 µL of phosphoric acid ($H_3PO_4$). Sealed ampoules are stored at 4 °C. The DOC concentration is determined in the laboratory by the high temperature combustion method using a Shimadzu TOC-5000 analyser. Standards are prepared with potassium hydrogen phthalate (Nacalao Tesque, Inc, Kioto, Japan). The mean concentration of triplicate injections of each sample (three in total) is calculated. The average analytical precision of the instrument was < 3 %.

**TSS / POC (total suspended solids and particulate organic Carbon)**

For TSS/POC pre-weighted glass fibre filters (GF/C) were used. Each filter was coded and stored in a clean Petri dish. The filtered volume was dependent on the particle load and concentration and type of organisms present in the water. The higher the total particle load in the sample, the smaller is the volume that can be filtered before the filter clogs. Practical volumes were between 100 and 1000 mL per sample.
After filtration the filter was rinsed with fresh water (MiliQ) to remove sea salt. Filters were dried overnight at 60 °C and allowed to cool in a vacuum exicator before weighing. The total amount of suspended solids was calculated from the weight increase of the filter and averaged for the three replicates (mg/L).
Next, the filter was combusted at 500°C (overnight) and allowed to cool in a vacuum exicator and weighted again. The POC is calculated from the weight decrease between this measurement and the TSS weight.

**Analytical determination of PERACLEAN® Ocean**

Two water samples were taken during each filling of the treated tank to get the initial concentrations during the actual filling of the tank. Sampling point was just behind the PERACLEAN® Ocean injection of the SEDNA®-system.
After filling of the tank and in the following days, samples were taken from the sampling point at tank 3 in the basement until the concentrations fell below the detection level.
At least three samples per day were taken in order to get sufficient data, evenly distributed over the time, to construct a degradation curve of the chemicals.

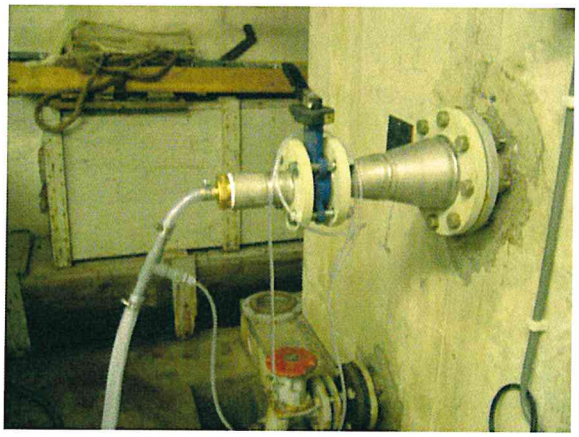

**Figure 4** :
Sampling point at tank 3

The amount of peracetic acid (PAA) and hydrogen peroxide ($H_2O_2$) was measured by photometric determination according to Pinkernell et al (Pinkernell et al. 1997) and Wagner & Ruck (Wagner and Ruck 1984), respectively. For each individual sample three measurements were done, and the average value was calculated. The detection limits for these methods are 0.5 mg/L PAA and 0.5 mg/L $H_2O_2$.

**Biology**

The majority, all of the large size fraction (> 50 µm) consists of zooplankton, while the majority of the small size (10 - 50 µm) fraction consists of phytoplankton. Organisms > 50 µm were retained as recommended in the G8 guidelines.
Samples for the 10 - 50 µm fraction were collected from the effluent of the Hydrobios net. These samples were then filtered over a 10 µm sieve and fixated.
A second set of samples for this size class was taken and not separated from the organisms < 10 µm in order to include the fate of the smaller sized plankton community as well and to avoid further damage of the plankton. The results of these samples are compared to the ones from the double filtered samples to evaluate the loss of organisms caused by processing the samples.

### Sample sizes

During the land-based tests containers from 1 to 1000 L were used for sampling and/or storage. Samples were taken continuously and evenly during the whole process of filling or emptying the ballast water tanks. These containers were thoroughly rinsed or heat treated prior to use. Samples for the human pathogens were taken in sterile (bar-coded) bottles provided by the bacterial test laboratory.

**Figure 5:**

500 L container with 50 µm Hydrobios net using a modified lid

### Organisms > 50 µm

The samples were pre-concentrated over a Hydrobios 50 µm net resulting in an end volume of approximately 100 to 200 mL. The samples are transferred to the lab directly after sampling and Neutral Red is added in a ratio that yields an end concentration of 1:50,000. Staining time is 2+ hours. Neutral Red stains living organisms (Crippen 1974; Fleming and Coughlan 1978) distinctively and quite rapidly (less than one hour, Fig. 6). Therefore the viability assessment remains unaffected by the possible death of organisms during the staining or during sample analysis.
It was assumed that dead but physically intact organisms will also be found. Consequently a detailed inspection of each intact individual is needed to assess viability. This includes the staining as well as the detection of internal (heart, gills) movement. Organisms which were not intact are assumed to be dead.

Neutral Red is a reliable staining method for all major groups of organisms but inconsistent staining was found for bivalves. For this latter group movement (including internal such as heart and gills for juvenile mussels) has to be used obligatorily to determine viability. This is dependent on the expertise of the person analyzing the samples. Therefore the same person analysed all samples.

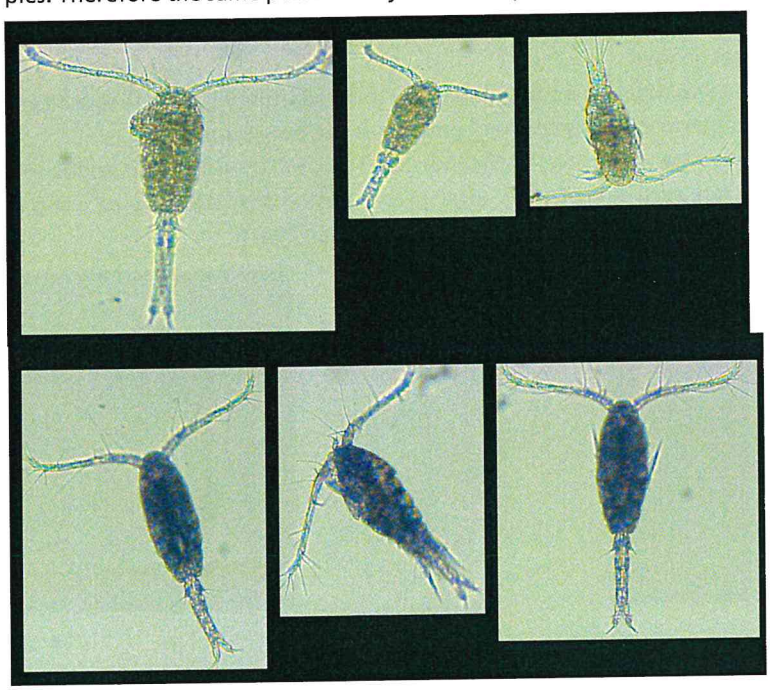

**Figure 6:** (top) dead copepods from a NR stained sample. (bottom) live copepods from a Neutral Red stained sample. FlowCam images kindly provided by N. Poulton Fluid Imaging Technologies.

The samples were analysed manually using a binocular with a 20x magnification for counting and up to 50x for species identification and measurements when necessary. For inter comparison a subset of samples was also analysed using a semi-automated tool (FlowCam, Fluid Imaging Technologies; Anonymous 2001, Fig. 6). Organisms need to be counted according to their size. Here organisms of 50 µm in minimum dimension are relevant. Several tests have shown that a single size bar is not efficient as viable organisms move in the counting chamber. Better results are achieved when the entire field of view is equipped with a size grid. The minimum dimension to measure will be adjusted to the specific organism groups. For minimum dimension measurements the "body" of the organism has been measured. Some examples are presented in figure. 7.

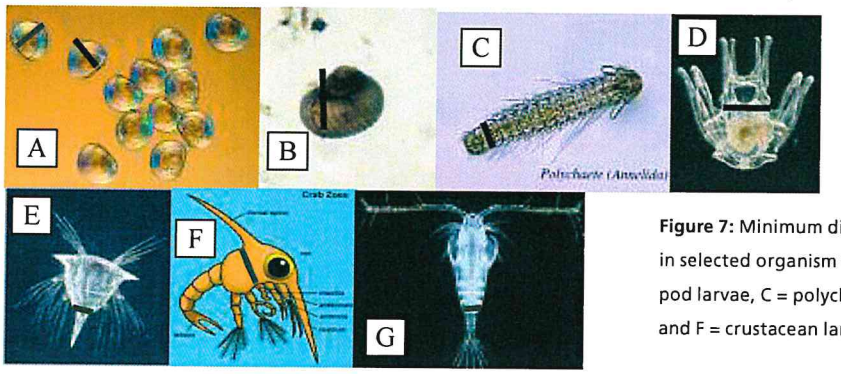


**Figure 7:** Minimum dimension measurements (black line) in selected organism types: A = bivalve larvae, B = gastropod larvae, C = polychaete, D = echinodermata larvae, E and F = crustacean larvae and G = copepod.

**Organisms < 50 µm**

Samples for visual inspection of species number and diversity were pre-concentrated using a sieve made of a Hydrobios 10 µm mesh net using the 50 µm prefiltered sample (effective size range is 10 – 50 µm). The retained organisms are flushed into 50 mL Greiner tubes using filtered seawater and fixed with Lugols solution. Sample analysis is conducted by microscopic count with an inverted microscope at 200x magnification (method by Utermöhl). Live-dead-separation in these samples is based on structural integrity of organisms. This method works for both zoo- and phytoplankton.
This size fraction was also covered by flow cytometry on basis of a single cell measurement (Veldhuis and Kraay 2000) and PAM fluorometry, as a bulk parameter (Schreiber et al. 1993), using the intact and undisturbed samples. Besides numbers and sizes these two methods can be used to assess the cell viability (Veldhuis et al. 2001; Veldhuis and Brussaard 2006) or in case of the PAM fluorometry also the photosynthetic efficiency of the phytoplankton.

Flow cytometry: For total organisms counts 3 mL of unfiltered sample water (reference and treated, each in triplicate) were analyzed using a calibrated flow cytometer. This yields the total number of particles (dead and live organisms and detritus) as well as their size range and the presence or absence of chlorophyll. For the counts exactly 1 mL was analysed.
The size of the plankton was determined by comparison to standardised beads (10 and 50 µm). These beads were also used as standards to calibrate the performance of the flow cytometer.
For organism viability testing, on the level of the individual cell, SYTOX Green was added to 1 mL of sample water (control and treated, each in triplicate). After 15 minutes samples are analysed using the flow cytometer for the presence of dead and/or life organisms (cf. Veldhuis et al, 2001).


**Figure**: 8(A) Epifluorescence microscopic picture of a life phytoplankton cell. The red signal is due to the presence of chlorophyll and (B) a dead phytoplankton cell with a yellow/green fluorescence of the nucleus after staining with SYTOX Green.

PAM fluorometry: The photochemical efficiency of photosystem II of phytoplankton (providing an estimate of the general health of the algae) can be addressed using a Pulse-Amplitude Modulated fluorometer (PAM-fluorometry) WALTZ- water PAM (Schreiber et al. 1993). For this 3 mL of unfiltered sample water (control and treated, each in triplicate) are filled into a glass cuvette and analysed using the Pulse-Amplitude Modulated fluorometer. The instrument was calibrated against filtered seawater and a healthy fast-growing population of phytoplankton.

Next to cell specific analysis chlorophyll *a* and its degradation products were determined to assess the fate of the whole phytoplankton community (Jeffrey et al. 1997). For this purpose water samples of 0.2 to 1.5 L (GF/F filters) were taken. The samples are frozen until further analysis. Filters are extracted with acetone (90%) for at least 24 hours and chlorophyll a concentration is determined spectrofluorometrically (standard procedures).

### Bacteria

The classical method for counting bacteria in many applications is based on plating on selective media. Unfortunately, for studies in the aquatic environment this approach is by far insufficient for various reasons (Gasol and Giorgio 2000). As a result total bacteria were now determined by flow cytometry, using DNA-specific stains to get a more accurate bacteria number. In addition samples were taken at discharge for specific human pathogens and heterotrophic bacteria using a plate method.
A 1.5 mL water sample is taken and pipetted in a Cryovial (in triplicate) and formaldehyde was added as a preservative. Samples were frozen and stored at -50 °C until further analysis.
Upon analysis the sample is allowed to thaw completely. A subsample of 100 µL is taken, diluted with a TE-buffer, and the nucleic acid dye PicoGreen (MP) was added. Within 5 to 15 minutes after the addition of the stain the sample is analysed using a flow cytometer (cf. Gasol and Giorgio 2000; Veldhuis et al. 1997). A known bacterial standard is used for calibration and counting.
The number of heterotrophic bacteria was determined using a plate method as the number of colony forming units (cfu's) after incubation of the water at intake and discharge according to an international standard (NEN-EN-ISO 6222:1999).

### Human pathogens

The samples for microbiological analysis were taken in special bottles of 600 mL and send to a special laboratory for further analysis. This laboratory is "Vitens laboratory bv" at Leeuwarden (accreditation certificate: NEN/ISO/IEC 17025; lab. no. L043). All analysis' are carried out according to NEN-EN-ISO standards.
These samples were sent to the laboratory immediately after sampling using a cooled transport container (4 °C). The analysis was carried out according NEN-EN-ISO 7899-2 for intestinal enterococci and NEN-EN-ISO 9308-1 for *E. coli* and related bacteria of the coli group as adopted for surface and waste water analysis in the Netherlands.

## Results

The present section is a compilation of all relevant information needed for Type Approval Certification tests according to the Guidelines (G8), but also includes some relevant results of experiments conducted to assess the environmental effect of the active substance and/or its by-products in the environment upon discharge. It should, however, be noted that detailed studies regarding potential toxicity according to standard procedures have also been carried out. The results were submitted to IMO/GESAMP-BWWG. Data are presented as averages or ranges separated for the two salinity regimes tested. In Annex 1, more detailed information on more specific parameters and a species list of organisms observed is presented.

The tests were carried out at two different salinity regimes (Tables 4 and 5) hereafter referred to as low and high salinity test series.

### Salinity

**Table 4:** Average salinity and temperature of water at intake during the low salinity tests of control and treated tanks for test runs 1 – 6.

| Test run | Salinity [PSU] | s.d. | Temperature °C |
|---|---|---|---|
| 1 - 6 | 21.4 | 2.3 | 13 - 17 |

To the low salinity test cycles brackish water from the NIOZ harbour was collected during low tide and fresh water, taken from Lake Ijssel was added to a maximum of 15% (v/v).

**Table 5:** Average salinity and temperature of water at intake during the high salinity tests of control and treated tanks for test runs 7 – 12.

| Test run | Salinity [PSU] | s.d. | Temperature °C |
|---|---|---|---|
| 7 - 12 | 33.5 | 1.4 | 18 - 20 |

To the high salinity test cycles coastal water from the North Sea was collected during high tide and a brine solution made from natural seasalt was added to a maximum of 4 % (v/v).

**Low salinity**

A summary of the results of the basic parameters (oxygen concentration, pH, TSS, POC and DOC) is presented in table 6 for the reference and treated water sample at intake and discharge.

**Table 6:** Oxygen, pH, TSS, POC and DOC concentrations of test series run at low salinity (6 test runs in total) at intake and discharge;
[1]: range of values;
[2]: average of reference and treated tank at intake (pump samples).

| Parameter | Intake | s.d. | Discharge (day 5) | s.d. | unit |
|---|---|---|---|---|---|
| O2 reference[1] | 7.4-12.7 | | 4.6-9.1 | | mg O2/L |
| O2 treated [1] | 23.3-28.3 | | 15.5-26.8 | | mg O2/L |
| pH reference | 8.31 | 0.16 | 8.27 | 0.16 | |
| pH treated | 6.42 | 0.18 | | | |
| TSS [2] | 121.3 | 32.6 | | | mg/L |
| POC[2] | 38.8 | 12.5 | 7.6 | 2.8 | mg/L |
| DOC reference | 5.0 | 1.39 | 4.1 | 0.68 | mg/L |
| DOC treated | 20.9 | 0.96 | 20.8 | 0.96 | mg/L |

Table 6 clearly demonstrates that as far as the basic parameters are concerned the values were in accordance with the criteria as indicated in the guidelines (G8, Table 1). As far as the oxygen values of the reference tanks are concerned values at intake were closely corresponding to a saturation values (100%) for the given salinity and temperature. Since the water temperature increased steadily during the test series values are presented as a range and not averaged. At discharge, on day 5, the oxygen concentration in the reference tank declined by ca. 30% compared to intake values. In contrast oxygen concentration in the treated tank was increased by as much as 300% as compared to the reference tank. This apparent super-saturation is partly an artefact caused by the applied Winkler method for $O_2$ measurements resulting in the dissociation of the $H_2O_2$ of PERACLEAN® Ocean (equation 1). Based on the initial concentration of added $H_2O_2$ of ca. 25 mg/L the theoretical increase in oxygen concentration according to equation 1 would be ca. 11.8 mg/L. In practice the measured values in the tank are 20 – 30% less than the theoretical concentration. Although not tested in detail this could be due to the simultaneous drop in pH altering the coloration of the Winkler reagents, out-gassing and oxygen respiration. After the required incubation period of 5 days the oxygen concentration dropped by as much as 40% but at discharge the oxygen level was still well above saturation.

Eq. 1. $$2H_2O_2 \rightarrow 2H_2O + O_2$$

The addition of PERACLEAN® Ocean caused a significant drop of the pH from an initial pH value of 8.31 of the seawater to 6.52 on average in the treated tanks after addition of the chemical (Table 6). This lowering of the pH is caused by the acetic acid present. At the end of the incubation the pH in reference tanks remained nearly constant but a slight increase was observed in the treated tank. As will be shown later the acidification of the water was reduced as a result of microbial degradation of the acetic acid or by diluting the discharge water with ambient seawater.

The total suspended solids (TSS) and particulate organic carbon (POC) concentrations were sufficiently high at intake but reduced at discharge (data not shown). For the reference tank this was mainly due to sedimentation but in the treated tank the mechanical treatment (hydrocyclone and filter) reduced the particle load significantly at intake.

Another striking difference between reference and treated tank was the concentration of dissolved organic carbon (DOC). At intake the natural (background) concentration of DOC of the Wadden Sea was ca. 5 mg/L but this increased by 4 fold in the treated tank (Table 6). As can be derived from equation 2 this was entirely due to the amount of organic compounds in the PERACLEAN® Ocean added.

Eq. 2 15 % PAA ⇨ 22.5 mg/L ⇨ 7.1 mg DOC /L
(1 mole PAA = 76.05 g including 24.02 g Carbon))

26.5 % HAc ⇨39.75 mg/L ⇨ 15.8 mg DOC /L
(1 mole HAC = 60.05 g including 24.02 g Carbon))

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in total as dissolved carbon 22.9 mg DOC/L

DOC estimation on addition of 150 mg/L PERACLEAN® Ocean immediately after injection, containing as potential carbon source 15 % Peracetic Acid (PAA) and 26.5 % Acetic acid (HAc).

In practice the theoretical value of 22.9 mg DOC /L originating from the active substance was not observed. At the end of the 5 days incubations the DOC content in the control tank dropped by ca. 20% but remained virtually constant in the treated tank. This implies that the discharge water contained a significant amount of acetic acid (HAc). The effect of this extra organic carbon source was therefore subject of additional studies (see section **Microbial** degradation of PERACLEAN® Ocean).

## High salinity

| Parameter | Intake | s.d. | Discharge (day 5) | s.d. | unit |
|---|---|---|---|---|---|
| O2 reference[1] | 7.1-8.2 | | 6.2-8.1 | | mg O2/L |
| O2 treated [1] | 23.5-28.8 | | 6.4-21.8 | | mg O2/L |
| pH reference | 8.13 | 0.05 | 8.08 | 0.04 | |
| pH treated | 6.27 | 0.07 | 6.29 | 0.06 | |
| TSS [2] | 11.7 | 4.4 | | | mg/L |
| POC[2] | 5.7 | 1.3 | | | mg/L |
| DOC reference | 3.0 | 0.18 | 2.8 | 0.25 | mg/L |
| DOC treated | 19.3 | 0.23 | 15.3 | 5.62 | mg/L |

**Table 7:** Oxygen, pH, TSS, POC and DOC concentrations of test series run at high salinity (6 test runs in total) at intake and discharge;

[1]: range of values;

[2]: average of reference and treated at intake (pump samples).

The results of the basic parameters at the high salinity range were, with a few exceptions, on the whole the same as for the low salinity range (Table 7). At intake the ambient water was saturated with oxygen and at discharge oxygen concentrations declined but they were still far from depleted. Partly because of the increase in seawater from the open North Sea sediment, POC and DOC values were much lower as compared to the low salinity test series. The TSS content increased at discharge which was mainly due to an increase in POC, which would be indicative of a potential increase in organisms since this was only observed in the treated tank and not in the refe-rence tank (POC control at day 5 declined to a value of 4.3 mg/L). The DOC concentration increased with a value of 16.3 mg/L, which was comparable to the value of 15.9 mg/L observed at low salinity. This value was again lower

than the expected value based on the theoretical composition of the active substance. Unlike the previous test series the DOC declined, by ca. 20% during the 5 days of incubation, which is indicative of active (bacterial) degradation of the HAc starting already prior to discharge.

## Biology

### Organisms > 50 µm

For the land based tests natural plankton was used and the required diversity of organisms (5 species of at least 3 different phyla) was easily fulfilled for all tests (Table 8). On average at least 10 different species of 4 to 5 different phyla were present in each sample (full details on species present see Annex 1).
Regarding the required minimal numbers of organisms per volume (Table 2), the values for the low salinity series (test run 1 -6) were, with exception of run 6, well above the requirements. On average numbers in the >50 µm size fraction were 50 times higher than the minimum required number of 100,000 $m^{-3}$. At the high salinity range the water at intake contained in two cases not sufficient numbers (test run 8 and 10). As explained earlier this was mainly due to the fact that natural seawater was used without any addition of organisms and that on average numbers of larger sized organisms are much lower in high saline water than brackish water. On discharge the number of organisms had dropped dramatically in the treated tank, to an average value for the low and high salinity test series of 0.12 and 0.05 organisms $m^{-3}$, respectively. In fact during most test series at direct inspection immediately after intake already no living organisms were observed. On average numbers in the treated tank were on discharge at day 5 were a factor 100 lower than the D2-standard of 10 organisms $m^{-3}$. In contrast numbers of organisms in the reference tank were still significantly above the G8 requirement and visual inspection and viability measurement indicated that these organisms were both intact and viable.

**Table 8:** Number of organisms > 50 µm at intake and on discharge at day 5 of the reference and treated tank. For test run 11 and 12 the same control was used, since this was a combined test run. All numbers are presented per $m^3$.

| total plankton > 50 micron | | | counts /m3 |
|---|---|---|---|
| low salinity | Intake | Reference | Treated |
| Test run | day 0 | day 5 | day 5 |
| 1 | 2300 E3 | 9.5 E3 | 0.7 |
| 2 | 15200 E3 | 15 E3 | 0 |
| 3 | 4600 E3 | 7.1 E3 | 0 |
| 4 | 4900 E3 | 7.1 E3 | 0 |
| 5 | 1400 E3 | 4.4 E3 | 0 |
| 6 | 77.5 E3 | 12.7 E3 | 0 |
| average | | 9.3 E3 ± 3.9 E3 | 0.12 |
| high salinity | | | |
| 7 | 103 E3 | 6.8 E3 | 0 |
| 8 | 60.0 E3 | 2.5 E3 | 0 |
| 9 | 113.2 E3 | 3.0 E3 | 0 |
| 10 | 57.2 E3 | 600 | 0.3 |
| 11 | 168 E3 | 1.5 E3 | 0 |
| 12 | 168 E3 | 1.5 E3 | 0 |
| average | | 2.7 E3 ± 2.2 E3 | 0.05 |

Additional analysis of some of the incubation experiments (see below for details) for organisms larger than 50 µm showed higher levels of survival (up to 4 times) in the incubated reference water as compared to the reference tank. However, in compliance with the results from the treated tanks no living organisms larger than 50 µm were found in the incubated treated water.

## Organisms 10 – 50 µm

This size class was dominated by phytoplankton, although heterotrophic organisms like ciliates and flagellates were occasionally present in high numbers. For that reason the flow cytometric analysis included chlorophyll *a* fluorescence as an extra selection parameter. Moreover, the photosynthetic efficiency of the phytoplankton community was measured, as a tool to determine the efficacy of the treatment more specifically, on this group of organisms. Finally, a 10 L subsample of both the reference and treated water was collected at intake and incubated under optimal growth conditions. Results of plankton numbers in the 10 – 50 µm of both reference and treated incubation are included in the table for comparison with the actual measurement of the tanks (Table 9). Table 9 shows that in the reference tank an incubation of 5 days, in the dark, resulted in a considerable reduction in the number of plankton in the 10 – 50 µm size range. At the low salinity test series this decline was on average 55% , as compared with intake, but during the high salinity test series this was much higher (81%). Nevertheless, the plankton community of the reference tanks still contained sufficient numbers of viable organisms to meet the G8 requirements. Moreover, the bottle incubations showed, with two exceptions (series 3 and 11/12) a considerable increase in numerical abundance within 5 days, i.e. presence of viable population of mainly phytoplankton. In contrast, treatment resulted in an efficient removal of plankton. Although after 5 days in the tanks some residual plankton cells were detected flow cytometrically, analysis of the viability using SYTOX Green, but also microscopic observation showed no viable cells anymore. In the incubated bottles of the treated ballast water even the detrimental particles were disintegrated after 5 days since virtually no particles within the given size range could be observed.

**Table 9:** Total plankton number of plankton in the 10 – 50 µm size range at intake, reference and treated tank, reference and treated incubated samples (Inc.) at day 5 during discharge. Runs 1 to 6 at low salinity range and 7 to 12 at high salinity range. Test run 11 and 12 share the same reference since this was a combined test run. All numbers are presented per ml as total counts or of viable cells (*).

| total plankton 10 - 50 µm | | | | counts/mL | | | * viable cells |
|---|---|---|---|---|---|---|---|
| low salinity | Intake | reference | | Treated | | | |
| Test run | day 0 | day 5 | Inc. T5 | day 5 total | day 5* | Inc. T5 | Inc. T5* |
| 1 | 1025 | 900 | 6070 | <1 | <0.1 | 0 | <0.01 |
| 2 | 2300 | 110 | 4720 | 18 | <0.1 | 1 | <0.01 |
| 3 | 2200 | 1310 | 222 | 6 | <0.1 | 0 | <0.01 |
| 4 | 1560 | 985 | 5060 | 12 | <0.1 | 3 | <0.01 |
| 5 | 600 | 321 | 4180 | 8 | <0.1 | 1 | <0.01 |
| 6 | 1100 | 350 | 7880 | 16 | <0.1 | 0 | <0.01 |
| average | 1464 | 663 | 4689 | 12 | <0.1 | 1 | <0.01 |
| high salinity | | | | | | | |
| 7 | 1242 | 810 | 2670 | 116 | <0.1 | 7 | <0.01 |
| 8 | 2500 | 570 | 4432 | 35 | <0.1 | 1 | <0.01 |
| 9 | 2079 | 114 | 3580 | 83 | <0.1 | 4 | <0.01 |
| 10 | 1000 | 180 | 2517 | 90 | <0.1 | 2 | <0.01 |
| 11 | 1841 | 201 | 933 | 48 | <0.1 | 1 | <0.01 |
| 12 | 1841 | 201 | 933 | 196 | <0.1 | 3 | <0.01 |
| average | 1751 | 346 | 2511 | 95 | <0.1 | 3 | <0.01 |

In order to have a more complete insight of the fate of all organisms, i.e. also the planktonic fraction < 10 µm in size diameter, a group of phytoplankton in the size range of ca. 6 µm was monitored in the control and treated water (Table 10). Although no clear criteria are, yet, defined for this size class it was clear that the SEDNA®-system was also effective in reducing organisms in this size range. Flow cytometry showed the presence of intact particles in the treated tanks even after 5 days. This was confirmed by classical analysis of the chlorophyll and associated pigment content (data not shown). Cells did loose their pigmentation and therefore the signal is rapidly masked by the detrital particles also present. With the high load of sediment, a clear differentiation of phytoplankton and other particles is less pronounced. For this size group the incubation is a far better approach to examine the residual growth potential of the plankton. Apparently the residual chlorophyll signal is disappearing completely since no plankton was observed in the target group after the incubation.

**Table 10:** Phytoplankton number <10 µm size range at intake, reference and treated tank, reference and treated incubated samples (Inc.) at day 5 during discharge. Runs 1 to 6 at low salinity range and 7 to 12 at high salinity range. Test run 11 and 12 share the same reference since this was a combined test run. All numbers are presented per mL as phytoplankton counts or of viable cells (*).

| phytoplankton <10 µm (group of ca 6 µm) | | | | | | | |
|---|---|---|---|---|---|---|---|
| low salinity | Intake | Reference | | Treated | | | |
| Test run | day 0 | day 5 | Inc. day 5 | day 5 | day 5* | Inc. T5 | Inc. day 5* |
| 1 | 8800 | 3100 | 60150 | 14400 | <0.1 | 0 | <0.01 |
| 2 | 53000 | 31800 | 65200 | 29000 | <0.1 | 6 | <0.01 |
| 3 | 13600 | 34600 | 31900 | 12500 | <0.1 | 3 | <0.01 |
| 4 | 23500 | 336 | 22750 | 2080 | <0.1 | 2 | <0.01 |
| 5 | 1400 | 76 | 15000 | 1720 | <0.1 | 8 | <0.01 |
| 6 | 1160 | 810 | 8400 | 330 | <0.1 | 3 | <0.01 |
| average | 16910 | 11787 | 33900 | 10005 | | 3.7 | <0.01 |
| high salinity | | | | | | | |
| 7 | 1900 | 2650 | 8315 | 448 | <0.1 | 3 | <0.01 |
| 8 | 8517 | 2179 | 13989 | 810 | <0.1 | 2 | <0.01 |
| 9 | 5865 | 2352 | 14680 | 680 | <0.1 | 3 | <0.01 |
| 10 | 1668 | 689 | 22291 | 771 | <0.1 | 0 | <0.01 |
| 11 | 15619 | 671 | 5687 | 67 | <0.1 | 1 | <0.01 |
| 12 | 15619 | 671 | 5687 | 391 | <0.1 | 1 | <0.01 |
| average | 8198 | 1535 | 11775 | 528 | | 1.7 | <0.01 |

## Photosynthetic efficiency

Another approach to gain insight in the physiological and growth response of the phytoplankton community is by measuring the photosynthetic efficiency (Fv/Fm) of the plankton cells. This can be done for the whole community or after filtration for different size classes when needed. For a clear understanding it must be noted that values of Fv/Fm > 0.4 are indicative of a healthy phytoplankton population; a Fv/Fm < 0.4 indicates that the phytoplankton community is experiencing severe stress and a value < 0.1 is typically observed in decaying phytoplankton populations.

Table 11 shows that during intake the whole phytoplankton community in the water, at the low as well as high salinity range was physiologically in a healthy condition, i.e. containing mostly photosynthetic active and therefore viable cells. Prolonged incubation in the dark (5 days of dark stress) of phytoplankton in the reference tanks resulted in a reduction in the photosynthetic efficiency, although some large differences in the magnitude of reduction was observed for the different test runs. Nevertheless, with exception of test run 5 the phytoplankton showed signs of stress but was certainly not entering the decaying stage. In contrast the photosynthetic efficiency of the phytoplankton in the samples incubated under optimal growth conditions showed a response varying from a mild stress to a healthy status (Reference Inc. day 5). This is not surprising since phytoplankton also increased in numerical abundance (Tables 9 and 10).

After passing the treatment system most of the phytoplankton community was immediately dead and as a result during discharge the Fv/Fm was typical of a decaying algal community. Also in the incubated sample there was no recovery of the phytoplankton population observed. It should be noted that the data as presented in Table 11 include the whole phytoplankton community and not exclusively the fraction > 10 µm.

**Table 11:** Photosynthetic efficiency (Fv/Fm) of the whole phytoplankton community (2 - > 50 µm size range) at intake, in the reference and treated tanks at discharge and in the incubated samples taken from the reference and treated tank after days of incubation. Runs 1 to 6 at low salinity range and 7 to 12 at high salinity range.

| Fv/Fm | | | | | |
|---|---|---|---|---|---|
| low salinity | | | | | |
| test run | Intake | Reference | | Treated | |
| | day 0 | day 5 | Inc. day 5 | day 5 | Inc. day5 |
| 1 | 0.5 | 0.21 | 0.41 | 0 | 0 |
| 2 | 0.52 | 0.50 | 0.34 | 0 | 0 |
| 3 | 0.44 | 0.22 | 0.32 | 0 | 0 |
| 4 | 0.47 | 0.14 | 0.50 | 0 | 0 |
| 5 | 0.5 | 0.09 | 0.29 | 0 | 0 |
| 6 | 0.71 | 0.56 | 0.57 | 0 | 0 |
| high salinity | | | | | |
| 7 | 0.63 | 0.18 | 0.56 | 0 | 0 |
| 8 | 0.61 | 0.33 | 0.51 | 0 | |
| 9 | 0.58 | 0.12 | 0.43 | 0.002 | 0 |
| 10 | 0.46 | 0.13 | 0.54 | 0 | 0 |
| 11 | 0.65 | 0.22 | 0.58 | 0 | 0 |
| 12 | 0.65 | 0.22 | 0.58 | 0.004 | 0 |

### Bacteria

For the microbial community the presence/absence of two types of human pathogens was monitored prior to and after treatment, while the response of the whole microbial community was also assessed.
Table 12 shows that even during intake the number of the target microorganisms was well below the standard as indicated in the D2-Standard. Only in two cases the number of human pathogens were above the detection limit (test run 7 and 8). The reason for this is that the NIOZ harbour is located in a pristine environment with little or no urban activity. Subsequently, also the number of human pathogens in the reference and treated tanks were below detection limit during discharge.

**Table 12:** Counts of the human pathogens *E. coli* and total enterococci at intake and during discharge of the reference and treated tank.

| Counts/mL | Intake (day 0) | | Reference (day 5) | | Treated (day 5) | |
|---|---|---|---|---|---|---|
| Test run | | | | | | |
| low salinity | *E. coli* | enterococci | *E. coli* | enterococci | *E. coli* | enterococci |
| 1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| 2 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| 3 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| 4 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| 5 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| 6 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| high salinity | | | | | | |
| 7 | 0.34 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| 8 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| 9 | <0.1 | 0.33 | <0.1 | <0.1 | <0.1 | <0.1 |
| 10 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| 11 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| 12 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |

In contrast to the nearly absence of human pathogens, the 'normal' marine microbial community was abundantly present. The total bacteria community is well studied in the Wadden Sea, using a method based on staining the

nucleic acid of the cells for many years. The currently observed numbers (Table 13) of numbers ranging from 3.4 to 7.6 $10^6$ $mL^{-1}$ are numbers typically present in spring and early summer. Of this total bacterial population only a very small fraction could be identified as heterotrophic ones using the plate assay method (10 to 140 per mL). Therefore, if only plating would be used as criteria for bacterial numbers this would result in a severe underestimation of the actual bacteria numbers. After 5 days of incubation total bacteria numbers declined in most, but not in all test runs in both the reference tank and in the incubated reference bottle. In general the incubation contained a slightly higher number of bacteria than the control tank. In a same manner the number of plated heterotrophs remained in the same order of magnitude at the low salinity range but declined to below the detection limit in the high salinity test series.
In the treated tanks and in the incubation bottles of this water the total bacteria numbers at discharge varied from the original numbers during intake in an inconsistent manner. In nearly half of the test runs there was an actual increase whereas in the others there was a decrease from the numbers at intake. These variations were nevertheless moderate and never exceeded the original values by more than a factor of 2.2. In terms of cultivable heterotrophic bacteria the treated water showed some remarkable differences with the water at intake and the reference. During the low salinity test runs the numbers of colony-forming heterotrophs remained unaltered in the first two test runs but increased significantly in the last 4 test series resulting in an increase of > 340 fold (test run 6). At all test runs at the high salinity range there was a very high increase in colony forming units, up to > 10000 fold increase, except for test run 12. As will be shown later in more detail this is caused by a fast growing population of heterotrophic bacteria using the residual acetic acid as their primary carbon and energy source. Growth of this bacterial group usually starts around day 5 with some variation in the original onset of the growth period.

**Table 13:** Total bacteria number and colony forming heterotrophs (numbers per ml) at intake and during discharge of the reference and treated tank as well as the total bacteria numbers in the incubated water samples. n.d.: not determined.

| | Intake | | Reference | | | Treated | | |
|---|---|---|---|---|---|---|---|---|
| Test run | total bact | colony | total bact | colony | total bact | total bact | colony | total bact |
| low salinity | day 0 | day 0 | day 5 | day 5 | Inc. day5 | day 5 | day 5 | Inc. day5 |
| 1 | 3.6E+06 | 10 | n.d. | 50 | n.d. | n.d. | 20 | n.d. |
| 2 | 3.4E+06 | 10 | 1.3E+06 | 20 | 3.6E+06 | 4.5E+06 | <10 | 4.9E+06 |
| 3 | 5.8E+06 | 20 | 7.1E+06 | 30 | 2.7E+06 | 8.2E+06 | 200 | 5.2E+06 |
| 4 | 7.6E+06 | 60 | 1.6E+06 | 50 | 3.0E+06 | 6.6E+06 | 190 | 7.2E+06 |
| 5 | 5.8E+06 | 20 | 1.7E+06 | 10 | 2.3E+06 | 6.9E+06 | 275 | 6.1E+06 |
| 6 | 5.2E+06 | <10 | 3.0E+06 | <10 | 3.1E+06 | 6.1E+06 | 3400 | 7.2E+06 |
| high salinity | | | | | | | | |
| 7 | 3.4E+06 | 20 | 2.6E+06 | <10 | 3.0E+06 | 6.1E+06 | >10000 | 4.0E+06 |
| 8 | 4.7E+06 | 10 | 1.4E+06 | <10 | 2.0E+06 | 4.6E+06 | >10000 | 3.8E+06 |
| 9 | 6.3E+06 | 140 | 0.9E+06 | 10 | 1.9E+06 | 2.8E+06 | >10000 | 3.4E+06 |
| 10 | 3.8E+06 | 10 | 1.2E+06 | <10 | 3.9E+06 | 1.5E+06 | 6800 | 3.0E+06 |
| 11 | 4.4E+06 | 10 | 9.5E+05 | <10 | 2.6E+06 | 4.1E+06 | 2500 | 4.2E+06 |
| 12 | 4.4E+06 | 10 | 9.5E+05 | <10 | n.d. | 5.8E+06 | 50 | 5.0E+06 |

## Environmental effects after discharge

### Microbial degradation of PERACLEAN® Ocean

Besides testing the SEDNA®-system for its efficiency to reduce the numbers of living organisms the system was also examined for it impact on the (marine) environment since it uses an active substance to kill organism in the second treatment step. The degradation of PAA was very rapid, usually within 24 h after addition (Table 14). PAA was transformed in the less reactive product acetic acid (Eq. 3). Together with the acetic acid already present in the formulation this resulted in an acidification of the treated water (Tables 6,7). Moreover, the added amount of acetic acid generates a large pool of labile organic carbon which could easily be decomposed by bacteria.

DOC caused by 150 mg/L PERACLEAN® Ocean can be calculated as follows:

Eq. 3
150 ppm PERACLEAN® Ocean with

| | | |
|---|---|---|
| 15% PAA | ⇨22.5 mg/L PAA ⇨ | 18.75 mg/L HAc |
| 26.5 % HAc ⇨ | | 39.75 mg/L HAc |
| in total | | 58.5 mg/L HAc |
| as dissolved carbon: | | 23.4 mg/L DOC |

(1 mole of HAc = 60,05 g and includes 24.02 g Carbon)

The PAA (peracetic acid), which is the reactive substance is transformed into HAc (hydrogen acetate = acetic acid). This dissolved organic pool of carbon can initially not be utilized by (heterotrophic) bacteria community because the additional presence of the bacteriostatic compound $H_2O_2$. $H_2O_2$ which remains present for several days (table 14) but a major fraction of the bacteria was also inactivated by the added PAA. Because of these two factors the regrowth of heterotrophic bacteria shows a significant lag phase of several days and starts effectively around day 5. It should be noted that mineralisation of the weak organic acid (HAc) by bacteria also results in an increase in pH.

**Table 14:** Temperature in the ballast tanks at intake and day 5 and degradation of the active ingredients PAA and $H_2O_2$ (h) after addition to detection limit (0.5 mg/L). n.d. not determined.

| test run | temperature day 0 | temperature day 5 | Time for PAA degradation [h] | Time for H2O2 degradation [h] |
|---|---|---|---|---|
| 1 | | | n.d. | n.d. |
| 2 | 13 | 14 | 16 | 114 |
| 3 | 15 | 15 | 18 | 74 |
| 4 | 16 | 16 | 18 | 90 |
| 5 | 18 | 16 | 20 | 100 |
| 6 | 16 | 17 | 18 | 106 |
| average | | | 18 | 97 |
| 7 | 18 | 18 | 16 | 90 |
| 8 | 18 | 18 | 18 | 96 |
| 9 | 20 | 20 | 18 | 68 |
| 10 | 21 | 20 | 8 | 70 |
| 11 | 21 | 19 | 8 | 54 |
| 12 | 21 | 19 | 16 | 90 |
| average | | | 14 | 78 |

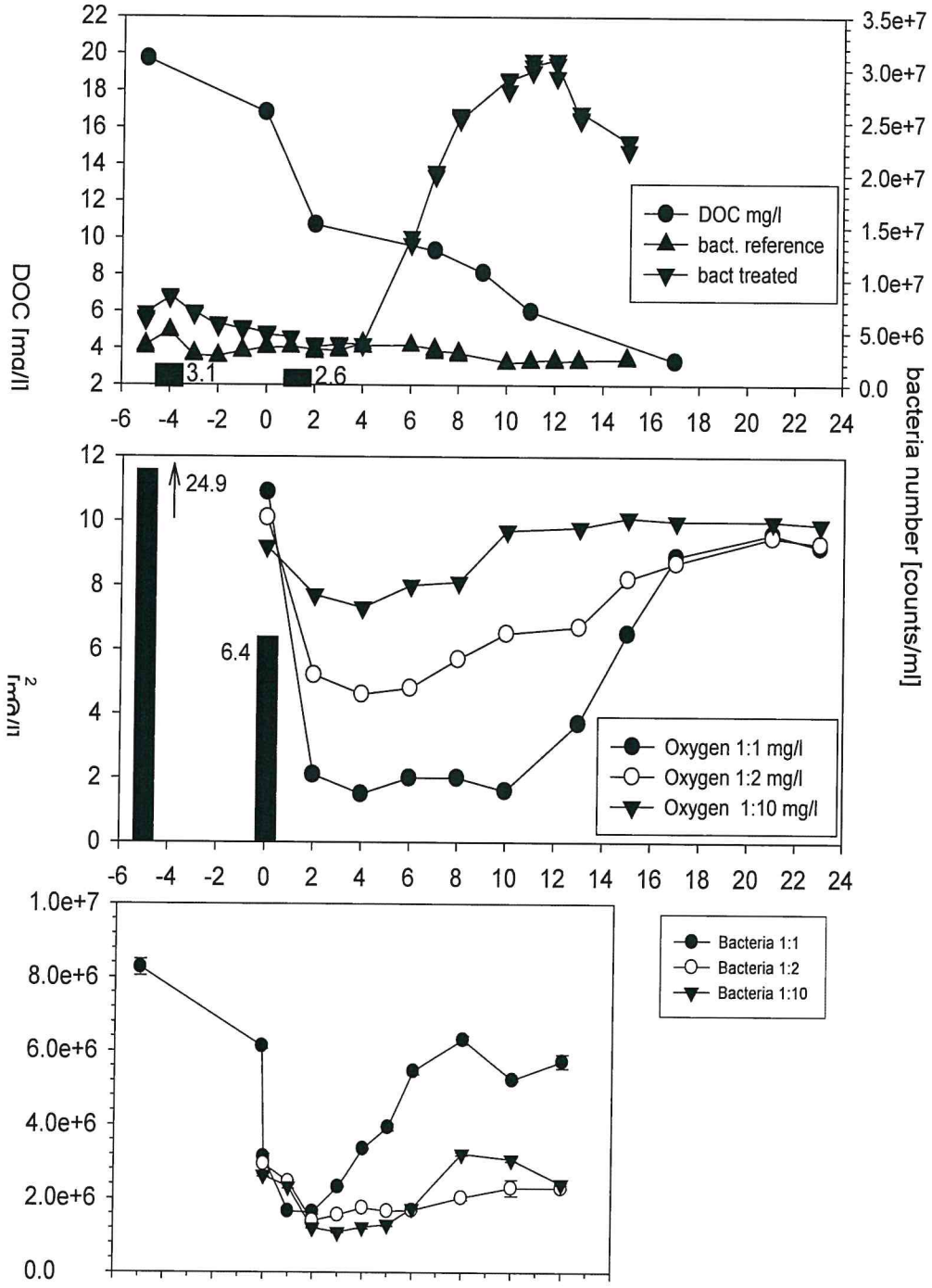


**Figure 9**: A) Total DOC concentration and total bacteria numbers in the reference and treated ballast water tank at intake (day -5), discharge (day 0) and after storage in a large tank (undiluted sample). Black bar is DOC concentration in reference tank at intake and discharge. B) oxygen concentration at intake and discharger (bars) in treated tanks and after diluting the discharge water in three concentrations with ambient seawater. The 100% saturation value of oxygen for the given temperature and salinity was 8.3 mg/L. C) bacteria numbers in the same containers (for first 12 days only).

The degradation of the HAc was monitored as the residual DOC concentration in a large volume tank over a period of 17 days (Fig. 9). The degradation during the initial 5 days incubation was usually low to moderate, on average no more than 10% of the HAc was mineralised during that period. The main component of the DOC content on discharge is therefore HAc. The degradation of this compound has been studied in a variety of experiments. Fig 9A shows that the complete bacterial degradation of HAc in a subsample (700 L) down to the ambient DOC concentration of the seawater takes about 17 days. In that period the bacteria numbers increase rapidly before declining again slightly.
In a second set of experiments the treated water was diluted during discharge, with freshly collected seawater in three dilution steps (1:1, 1:2 and 1:10 v/v) and oxygen and bacteria numbers were measured during a 23 and 12 days period, respectively (Fig. 9B,C). The dilution of the treated water with the water from the NIOZ harbour resulted in an initial increase in the oxygen concentration to values above the saturation value for the given temperature and salinity. In the following 4 days the oxygen concentration declined rapidly, but this decline varied with the initial dilution step. In the least diluted tank (1:1) the oxygen value dropped to a value of 15% of the normal saturation level and remained at that level for another 6 days. In the less diluted tanks oxygen levels did not drop that much and in all tanks a recovery was observed after day 10 to values above 100% saturation values. This was basically due to photosynthetic activity of the phytoplankton community (data not shown). In terms of bacteria numbers only the least diluted tanks showed a significant increase in numbers, thereby explaining the concurrent drop in oxygen concentration. In the other two tanks there was only a moderate increase in bacteria numbers and therefore also a moderate drop in oxygen concentration.

## 7. Discussion of results

The presented data show that the experimental design, type of test protocols used and additional experiments provide a solid data base of information on the performance of the SEDNA®-system BWT installation under semi *in situ* conditions. The outcome was in a way not surprising since prior to testing according to the guidelines (Anonymous, 2005) of the IMO some experience had been gained with testing the SEDNA®-system for its efficacy (Veldhuis et al. 2006). During the testing period we encountered three major issues which need urgent attention.

1. The number of organisms in the > 50 µm fraction, and to some extend also in the 10 – 50 µm size range, can not (easily) be met in the salinity range > 32 PSU. The addition of cultured organisms is very difficult and poses a variety of problems. With increasing salinity the seawater will have the characteristics of typical open ocean water. This implies low to extremely low numbers of the organisms in the above indicated size ranges. For that reason we have extended our focus to the organisms < 10 µm since there is no biologically relevant reasoning why tests should exclude this size fraction. As demonstrated during these tests the proper tools and technologies are available to test this size fraction for its presence and viability status.
2. Short- and long-term incubations should be an integrated part of land-base testing. While the large tank trials offer a more realistic scenario, sampling, sample handling and analysis is still time-consuming and labour-intensive. In particular the microscopic observations are a tedious task and the high load of sediments can be hampering proper inspection of the samples. To some extend semi-automated tools like flow cytometry and flow cam reduce this 'human' error but do not completely solve it. An elegant approach to test the efficacy of the ballast water system is to examine the remaining viability of organisms after treatment. The best way to do so is to transfer a sufficiently large volume of water under optimal growth conditions for plankton and to monitor for abundance and viability for a considerable period.
3. Testing for the presence of human pathogens strongly depends on the natural abundance of the microbes in the natural environment. Since these pathogens cannot be supplemented for safety reasons accurate testing is therefore not possible. Moreover, viability tests of the total bacteria community showed that not all bacteria were effectively killed. Therefore, at least in theory the human pathogens would remain a potential risk. This is a factor of concern for the land-based tests as well as for the ship-board trials when the ship remains in fairly clean ports for intake and discharge. In the case of PERACLEAN Ocean its active compound (PAA) has been evaluated for total and fecal coliforms (*E. coli:* Antonelli et al. 2006 ) as well as with *V. cholerea* (Baldry et al. 1995) and in both studies proven to be a effective method of disinfection.

## 8. Evaluation of results

The present land-based tests with the SEDNA®-system show that the BWT installation and the management plan performs in accordance with the IMO Convention as stated in the Standard-D2 and according to the criteria for testing as indicated in the G8. The system has been thoroughly tested for a considerable period (March till July) covering a full spring season with a huge biodiversity and organism load. This period also included a large bloom

of the mucus containing phytoplankter *Phaeocystis globosa*, an organisms which is known to clog nets of all sorts of sizes and certainly also the 50 µm size used in the present filter. In fact the system performed even better than the D2-standard. For both size classes of organisms the observed residual numbers of viable organisms on discharge were at least 100 fold lower than the Standard-D2 allows. In addition also the plankton fraction < 10 µm was effectively killed by the system and no recovery was observed for up to 18 days. In general the organism decompose completely and contribute to the pool DOC but can mainly be traced as a amorphous fluffy substance free-floating in the water or precipitating to the bottom of the tanks.

The fact that this system used an active substance, an organic acid combined with active oxygen, resulted in a detailed examination of the (residual) effects of these compounds on the organisms at intake but also after discharge. The life time of the active ingredients is rather short, less than 24 h for PAA and a few days for $H_2O_2$, but this period increases at very low temperatures (Lafontaine and Despatie 2006). Although this may result in a discharge of low amounts of active compounds in the environment, these ingredients are not uncommon in the aquatic environment, although natural concentrations are usually lower. The degradation product of the active ingredient of PAA is HAc, also a common organic acid in the aquatic environment although concentrations are usually several orders of magnitude lower. Because of its acidic nature the pH is reduced, not fully buffered by the buffering capacity of the seawater. In addition during microbial degradation of HAc oxygen will be utilised resulting in a reduced oxygen concentration of the water. These effects are only temporarily and there is a full recovery within a period of a few days. The bacterial degradation of the organic acid is completed within several days, resulting in a temporal increase in microbial biomass. The decline in oxygen concentration is also temporarily and true oxygen depletion (anoxia) was never observed. In contrast signs of true oxygen depletion were observed in the reference tanks as could be derived from the typical smell of $H_2S$ during cleaning of the tanks.

In conclusion, the present configuration of the SEDNA®-system and its active substance PERACLEAN® Ocean offers a reliable and environmentally safe cleaning of the ballast water resulting in organism numbers well below the Standard of the IMO Regulation-D2.

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Appendix 1:

| **Phytoplankton Marsdiep April-July 2007** | +: present but rare, ++: present; +++: dominant; |
|---|---|
| **data provided by Jolanda van Iperen** | ++++: very abundant; +++++: massively present |

| group | species name | relative dominance |
|---|---|---|
| autotrophic flagellate | *Phaeocystis globosa* colony cell | +++++ |
| | *Phaeocystis globosa* colonies < 50 µm | ++++ |
| | *Phaeocystis globosa colonies > 50 µm* | +++++ |
| autotrophic flagellate | *Phaeocystis globosa* flagellate cell | +++++ |
| heterotrophic flagellate | parasite cyst of *Ochromonas* group | ++++ |
| diatom pennate | *Pseudonitzschia delicatissima* group | ++++ |
| heterotrophic flagellate | heterotrophic flagellate indet. <10 µm | ++++ |
| autotrophic flagellate | Prymnesiales indet. <10 µm | ++++ |
| diatom centricate | *Thalassiosira* spp. 10 µm | ++++ |
| diatom centricate | *Chaetoceros socialis* | ++++ |
| autotrophic flagellate | *Hemiselmis* group | +++ |
| diatom centricate | *Skeletonema "costatum"* group | +++ |
| autotrophic flagellate | *Plagioselmis* group | +++ |
| heterotrophic flagellate | *Paulinella* spp. | +++ |
| heterotrophic flagellate | heterotrophic flagellate indet. 10-30 µm | ++ |
| autotrophic flagellate | *Pyramimonas* spp. <10 µm | ++ |
| heterotrophic flagellate | Choanoflagellatea indet. | ++ |
| hetero/auto flagellate | Cryptophyceae "light" group | ++ |
| autotrophic flagellate | *Teleaulax acuta* group | ++ |
| heterotrophic dinoflagellate | *Oxyrrhis marina* | ++ |
| diatom centricate | *Leptocylindrus minimus* | ++ |
| diatom centricate | *Minutocellus* group | ++ |
| diatom centricate | *Thalassiosira* spp. 10-30 µm | ++ |
| heterotrophic dinoflagellate | *Katodinium glaucum* | ++ |
| diatom centricate | *Guinardia delicatula* | ++ |
| freshwater green alga | *Pediastrum* spp. | ++ |
| heterotrophic flagellate | *Ciliophrys* group | ++ |
| autotrophic flagellate | Prasinophyceae indet. <10 µm | ++ |
| hetero/auto dinoflagellate | Gymnodiniaceae indet. 10-30 µm | ++ |
| diatom centricate | *Leptocylindrus danicus* | ++ |
| diatom centricate | *Brockmanniella brockmannii* | ++ |
| diatom centricate | *Cerataulina pelagica* | ++ |
| autotrophic flagellate | autotrophic flagellate indet. <10 µm | ++ |
| diatom centricate | *Chaetoceros* spp. <10 µm solitairy cells | + |
| autotrophic flagellate | Chlorophyceae indet. | + |
| heterotrophic flagellate | Chrysophyceae indet. | + |
| freshwater green alga | *Crucigenia* spp. | + |
| heterotrophic flagellate | *Telonema* spp. | + |
| freshwater green alga | Chlorophyta fresh indet. | + |
| diatom centricate | *Skeletonema "costatum"* lenses | + |
| freshwater green alga | *Oocystis* spp. | + |
| diatom pennate | *Asterionellopsis glacialis* | + |
| autotrophic flagellate | *Rhodomonas* group | + |
| diatom centricate | *Odontella aurita* | + |
| heterotrophic flagellate | *Bodo* group | + |
| diatom centricate | *Chaetoceros* spp. >10 µm no bristle chloroplast | + |

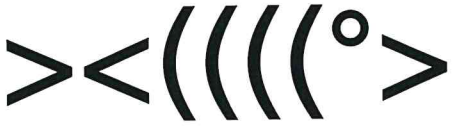
Species list zooplankton and some larger (atypical) phytoplankton

| Phylum | Class | Subclass, Order, etc. | species no. | Identified genera | Most likely present |
|---|---|---|---|---|---|
| Sarcomastigophora | | Dinoflagellida | 2 | Noctiluca, Protoperidinium | |
| Bacillariophyceae | | | 3+ | Bidulphia, Coscinodiscus | |
| Cnidaria | Hydrozoa | | 2+ | Obelia | |
| | Scyphozoa | | 2 | Aurelia, Cyanea | |
| Ctenophora | | | 2+ | | *Pleurobrachia, Beroe, Mnemiopsis* |
| Nemathelminthes | Rotatoria | | 1+ | | *Asplanchna* |
| | Nematoda | | 1+ | | |
| Annelida | Polychaeta | | 2+ | | |
| Arthropoda | Crustacea | Order Calanoida | 4+ | Temora, Acartia, Centropages, Calanus and/or Pseudocalanus | *Oithona* |
| | | Order Harpacticoida | 2+ | | *Tigriopus* |
| | | Subclass Cirripedia | 1+ | | *Semibalanus* |
| | | Suborder Cladocera | 2 | Podon, Evadne | |
| | | Subclass Malacostraca | 2+ | Carcinus (zoea larvae) | |
| Mollusca | Gastropoda | | 1+ | | *Littorina* |
| | | | 2+ | Cerastoderma | *Mya* |
| Echinodermata | Ophiuroidea and/or Echinoidea | | 2+ | | *Ophiothrix, Echinocardium* |
| Urochordata | Larvacea | | 1 | Oikopleura | |
| Minimum number of species encountered (10 phyla): | | | 32 | | |

# Final report of the shipboard testing of the SEDNA®-System, for Type Approval according to Regulation D-2 and the relevant IMO Guideline

**(June – December 2007)**

**Shipboard testing on board OOCL Finland**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# Final report of the shipboard testing of the SEDNA®-System, for Type Approval according to Regulation D-2 and the relevant IMO Guideline

**(June – December 2007)**

**Shipboard testing on board OOCL Finland**

Dr. Stephan Gollasch

Dr. Marcel Veldhuis

Frank Fuhr, Dipl.-Biol.

## Shipboard testing on board OOCL Finland

### General

The shipboard tests for final approval took place on three voyages of the container vessel OOCL Finland in 2007. The details of the vessel are outlined in the following tables 1 - 3.

**Table 1:** Main dimensions of the test vessel

| | |
|---|---|
| Length overall Loa | 134.44 m |
| Length b. perpendiculars Lpp | 124.41 m |
| Breadth (mld.) B | 22.50 m |
| Depth to main deck (mld.) D | 11.30m / 14.00 m |
| Scantling draft (mld.) dmax | 8.712 m |

**Table 2:** Ballast water capacity of the test vessel

| | |
|---|---|
| Total ballast water capacity | 3932 m³ |
| Extra ballast water capacity | 0 |
| Number of segregated ballast water tanks | 21 |
| Number of other tanks used for ballast water | 0 |
| Number of ballast water pumps | 2 |
| Capacity of ballast water pumps (each) | 225 m³/h |

**Table 3:** Tonnage measurement of the test vessel

| | | |
|---|---|---|
| Deadweight tdw | abt. 11250 | t |
| Gross Tonnage | 9981 | GT |
| Net Tonnage | 6006 | NT |

Description of the ships ballast water system and the sampling regime

The test ship is trading in short sea shipping, sailing in the Baltic and North Seas region. Due to the nature of the trade (containers), it was difficult to identify a suitable set of ballast water tanks for the G8 certification tests. According to the requirements of the G8 guideline for shipboard testing, both ballast water tanks, the control tank and the treated tank, must be filled and emptied in total, either in parallel or in sequence. However, the stability of small container ships like the test ship allow only for ballast water operations in accordance with the load conditions. This normally includes the partly filling and/or emptying of ballast water tanks. The double bottom tanks no. 2 P and no. 2 Stb were identified as the only suitable tanks for the tests. Both tanks had a volume of 70m³. Because of this small volume, the samples could be collected over each entire filling and emptying of the tanks, respectively. This resulted in a much more accurate measurement of organism densities than just sub-samples from parts of the ballast water operation.

The installed SEDNA®-system had a capacity to match the capacity of the ballast water pumps. Both systems were installed on deck in a special 40-foot container. The SEDNA®-system was fully implemented and integrated into the ballast system of the ship. A ballast water management plan was developed especially for this ship, with the ballast water treatment being the preferred ballast water management option. The Ballast water management plan as a whole was certified by Germanischer Lloyd (GL).

Tests were conducted by Dr. S. Gollasch (GoConsult, Hamburg, Germany). In total five consecutive, valid test runs were undertaken during the test period from June 2007 to December 2007. It should be noted that during the fifth test run carried out from 05.12.2007 to 06.12.2007, the treated ballast water also complied with the water quality standard D-2. However, organism numbers upon discharge for the control tank were below 10-times the D-2 standard (see table 10 for details). This was due to the very low number of organisms encountered at intake, as a result of sampling in winter with little plankton present. Since no elevated level of mortality compared to the other test runs was observed in the control tank of the fifth test run, the results were also taken into account for the evaluation of the systems performance.
During each of the test runs samples were collected by using multiple Hydrobios nets in parallel. The total volume of each of the samples in summarized in table 4.

**Table 4:** Details of the test runs. The volumes given for the samples represent single samples for the control tank and the treated tank upon uptake, as well as triplicate samples for the treated tank upon discharge

| BW-Operation | Test-cycle no. 1 | | Test-cycle no. 2 | | Test-cycle no. 3 | | Test-cycle no. 4 | | Test-cycle no. 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Date BW-uptake | 16.06.07 | | 29.08.07 | | 02.09.07 | | 28.11.07 | | 05.12.07 | |
| Location region | leaving Gävle | | east of Bornholm | | Kotka | | near Texel | | west of St. Petersburg | |
| | control | treated | control | treated | control | treated | control | treated | control | treated |
| Sample vol. [l] | 1.232 | 1.534 | 1.290 | 1.429 | 1.141 | 1.207 | 1.010 | 1.012 | 1.438 | 1.672 |
| Retention time | 32 hrs | | 58 hrs | | 42 hrs | | 31 hrs | | 30 hrs | |
| Date BW-discharge | 18.06.07 | | 01.09.07 | | 04.09.07 | | 30.11.07 | | 06.12.07 | |
| Location region | before Kiel Canal | | Kotka | | St. Petersburg | | west of Bornholm | | before Kiel Canal | |
| | control | treated | control | treated | control | treated | control | treated | control | treated |
| Sample vol. | 2.117 | 2.561<br>2.914<br>2.416 | 3.071 | 2.628<br>2.542<br>1.238 | 1.859 | 1.898<br>1.887<br>1.658 | 10.17 | 1.070<br>1.004<br>1.017 | 2.220 | 2.387<br>2.383<br>2.563 |

Plankton organisms larger than 50 µm in minimum dimension were analysed on board directly after sampling. Human pathogens were prepared onboard for later analysis by Vitens BV in Leeuwarden, The Netherlands and all other samples were processed at NIOZ, Texel, The Netherlands. Procedures were followed according to the test protocol. Like in the North Sea region the human pathogen *Vibrio cholera* (O1 and O139) is not present in the Baltic Sea (e.g. Hökeberg 2007). Due to the reliable information about the absence of *V. cholera*, in combination with the problems associated with testing for *V. cholera* on a routine basis, it was decided not to perform extra tests for *V. cholera*. Furthermore the efficacy of the active components, peracetic acid and hydrogen peroxide, against *V. cholera* has been shown before (Baldry et al 1995, Sagripanti et al 1997).

## Results

The basic set of abiotic parameters analysed during the different tests runs are summarised in table 5.

**Table 5:** Physical parameters of the source water (temperature, salinity, total suspended solids and particulate organic carbon) according to the G8 Guidelines. The values represent single samples for the control tank and the treated tank upon uptake, as well as the average of the triplicate samples for the treated tank upon discharge. b.d.l.: below detection limit ( about 2 to 5 mg/l due to transport and handling)

| BW-Operation | Test-cycle no. 1 | | Test-cycle no. 2 | | Test-cycle no. 3 | | Test-cycle no. 4 | | Test-cycle no. 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Uptake | 16.06.07 | | 29.08.07 | | 02.09.07 | | 28.11.07 | | 05.12.07 | |
| Location region | leaving Gävle | | east of Bornholm | | Kotka | | near Texel | | west of St. Petersburg | |
| | control | treated | control | treated | control | treated | control | treated | control | treated |
| T°C | 11,0 | 10,6 | 15,9 | 15,6 | 15,8 | 15,9 | 9,9 | 9,9 | 3,9 | 3,8 |
| Sal. | 9,11 | 8,92 | 5,2 | 5,2 | 5,3 | 5,3 | 20,5 | 20,5 | 5,2 | 5,1 |
| TSS [mg/l] | ~ 30 | ~ 30 | ~ 40 | ~ 40 | ~ 50 | ~ 40 | ~ 20 | ~ 20 | ~ 40 | ~ 40 |
| POC [mg/l] | ~ 10 | ~ 10 | ~ 15 | ~ 15 | ~ 30 | ~ 20 | b.d.l. | b.d.l. | ~ 25 | ~ 25 |
| Discharge | 18.06.07 | | 01.09.07 | | 04.09.07 | | 30.11.07 | | 06.12.07 | |
| Location region | before Kiel Canal | | Kotka | | St. Petersburg | | west of Bornholm | | before Kiel Ccanal | |
| | control | treated | control | treated | control | treated | control | treated | control | treated |
| T°C | 14,6 | 14,4 | 14,3 | 14,1 | 14,4 | 14,6 | 8,4 | 7,4 | 7,1 | 7,0 |
| Sal. | 8,93 | 8,88 | 5,3 | 5,1 | 5,4 | 5,2 | 22,0 | 23,0 | 4,8 | 5,3 |
| TSS [mg/l] | ~ 10 | ~ 10 | ~ 20 | ~ 10 | ~ 10 | ~ 10 | ~ 20 | ~ 8 | ~ 20 | ~ 10 |
| POC [mg/l] | b.d.l. | b.d.l. | ~ 8 | b.d.l. | b.d.l. | b.d.l. | ~ 10 | b.d.l. | ~ 15 | b.d.l. |

The biological results of the shipboard tests are summarized in the tables 6 to 10. The results show that the SEDNA® - ballast water treatment system complied with the standards in Regulation D-2 for all tested groups during all tests. This is consistent with the results of the land based tests. There are two remarks to be made related to the results. Firstly, the low number of organisms in control tank at discharge during the fifth test run (see above). Secondly, due to technical problems no results on the human pathogens are available for the second test cycle (Table 7).

**Table 6:** Results first test cycle from 16.06. (Gävle) to 18.06.2007 (Kiel Canal). Uptake: Number of organisms during BW-uptake, Discharge: Number of organisms during BW-discharge, n.d.: not determined

| Group | Control | | Treated | |
|---|---|---|---|---|
| | uptake | discharge | uptake | discharge |
| > 50µm / m³ | 1.623 | 387 | 1.245 | 1,1 |
| 10µm-50µm / ml | 576 | 185 | 495 | <0.1 |
| bacteria * 10^4/ ml* | 143 | 168 | 148 | 231 |
| *E. coli* [cfu / ml] | <0.1 | 0,16 | <0.1 | <0.1 |
| Enterococcus [cfu / ml] | <0.1 | <0.1 | <0.1 | <0.1 |
| *Vibrio cholera* [cfu / ml] | n.d. | n.d. | n.d. | n.d. |
| total heterotrophic bacteria / ml | 300 | 190 | 120 | 5 |

* these are total bacteria counts

**Table 7:** Results second test cycle from 29.08. (Bornholm) to 01.09.2007 (Kotka). Uptake: Number of organisms during BW-uptake, Discharge: Number of organisms during BW-discharge, n.d.: Not determined

| Group | Control | | Treated | |
|---|---|---|---|---|
| | uptake | discharge | uptake | discharge |
| > 50µm / m³ | 11.757 | 1.791 | 6.786 | 0,0 |
| 10µm-50µm / ml | 1.784 | 1123 | 1.254 | <0.1 |
| bacteria * 10^4/ ml* | 188 | 172 | 174 | 202 |
| *E. coli* [cfu / ml] | n.d. | n.d. | n.d. | n.d. |
| Enterococcus [cfu / ml] | n.d. | n.d. | n.d. | n.d. |
| *Vibrio cholera* [cfu / ml] | n.d. | n.d. | n.d. | n.d. |
| total heterotrophic bacteria / ml | n.d. | n.d. | n.d. | n.d. |

* these are total bacteria counts

**Table 8:** Results third test cycle from 02.09. (Kotka) to 03.09.2007 (St. Petersburg). Uptake: Number of organisms during BW-uptake, Discharge: Number of organisms during BW-discharge, n.d.: Not determined

| Group | Control | | Treated | |
|---|---|---|---|---|
| | uptake | discharge | uptake | discharge |
| > 50µm / m³ | 4.981 | 395 | 2.845 | 0,0 |
| 10µm-50µm / ml | 785 | 382 | 764 | <0.1 |
| bacteria * 10^4/ ml* | 201 | 236 | 245 | 211 |
| *E. coli* [cfu / ml] | <0.1 | <0.1 | <0.1 | <0.1 |
| Enterococcus [cfu / ml] | <0.1 | <0.1 | <0.1 | <0.1 |
| *Vibrio cholerea* [cfu / ml] | n.d. | n.d. | n.d. | n.d. |
| total heterotrophic bacteria / ml | >10000 | >10000 | 30 | <10 |

* these are total bacteria counts

**Table 9:** Results fourth test cycle from 28.11. to 30.11.2007. Uptake (To): Number of organisms during BW-uptake, Discharge (Ta): Number of organisms during BW-discharge, n.d.: Not determined

| Group | Control | | Treated | |
|---|---|---|---|---|
| | uptake (To) | discharge (Ta) | uptake (To) | discharge (Ta) |
| > 50µm / m³ | 624 | 324 | 919 | 0,0 |
| 10µm-50µm / ml | 184 | 166 | 179 | <0.1 |
| bacteria * 10^4/ ml* | 385 | 295 | 315 | 198 |
| E. coli [cfu / ml] | <0.1 | <0.1 | <0.1 | <0.1 |
| Enterococcus [cfu / ml] | <0.1 | <0.1 | <0.1 | <0.1 |
| *Vibrio cholerea* [cfu / ml] | n.d. | n.d. | n.d. | n.d. |
| total heterotrophic bacteria / ml | 1300 | <10 | 700 | 15 |

* these are total bacteria counts

Table 10: Results fifth test cycle from 05.12. to 06.12.2007. Uptake (To): Number of organisms during BW-uptake, Discharge (Ta): Number of organisms during BW-discharge, n.d.: Not determined

| Group | Control | | Treated | |
|---|---|---|---|---|
| | uptake (To) | discharge (Ta) | uptake (To) | discharge (Ta) |
| > 50µm / m³ | 209 | 20 | 224 | 0,0 |
| 10µm-50µm / ml | 41 | 39 | 39 | <0.1 |
| bacteria * 10^4/ ml* | 346 | 248 | 327 | 211 |
| E. coli [cfu / ml] | <0.1 | <0.1 | <0.1 | <0.1 |
| Enterococcus [cfu / ml] | <0.1 | <0.1 | <0.1 | <0.1 |
| Vibrio cholerea [cfu / ml] | n.d. | n.d. | n.d. | n.d. |
| total heterotrophic bacteria / ml | 140 | >10000 | <10 | <10 |

* these are total bacteria counts

**Discussion of results**

The ship board tests with the SEDNA®-system showed that the ballast water treatment (BWT) installation and the management plan performed in accordance with the standards as stated in Regulation D-2 of the IMO Ballast Water Management Convention and further meets the test criteria as indicated in the IMO Guideline G8 for type approval tests of BWTS. The system has been thoroughly tested for a considerable period (June untill December) in different environmental conditions and during different seasons. During these tests the SEDNA®-system has proven to be seaworthy and highly effective. The performance onboard the OOCL Finland was consistent with results obtained during land based tests. The slightly higher number of surviving organisms found in comparison to the land based tests can be attributed to residual, untreated water remaining present in the ballast pipes of the ship. This is due to the fact that for shipboard testing not all ballast tanks were treated nor that the BWT system is used continuously. This is most likely also the reason for the observed variations of numbers at intake, and especially for bacteria at discharge of the control tank during the third test run. Unless the ballast water of the whole ship is treated this will remain a problem and therefore a factor to consider.

Like for the land based test the shipboard tests also included inspection for all size ranges of plankton and not only the size classes relevant to Regulation D-2. Although not presented in this report also in the size class <10 µm no living (phyto)plankton was observed in the treated water upon discharge. Also the number of both total and heterotrophic bacteria dropped significantly in the treated water as compared with the intake and control water. In this respect the results differed slightly with those of the land-based tests showing occasionally an increase in total and heterotrophic bacteria in the treated water. This was mainly due to the fact that

the remaining acetic acid of the PERACLEAN Ocean acted as a substrate for growth of bacteria which apparently did not occurred during the ship tests during the given ballasting and deballasting periods.

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