

BALLAST WATER ANALYSIS AND HEAT TREATMENT USING WASTE HEAT RECOVERY SYSTEMS ON BOARD SHIPS

Yanran Cao¹, Vilmar Æsøy² and Anne Stene¹

¹Faculty of Life Sciences

²Faculty of Maritime Technology and Operations

Aalesund University College

N-6025, Aalesund, Norway

E-mail: {yaca, aste, ve}@hials.no

KEYWORDS

Ballast water, micro organisms, heat treatment,

ABSTRACT

Ballast water contains a variety of organisms including bacteria, viruses and the adult and larval stages of the many marine and coastal plants and animals. As such, it poses serious ecological, economic and health problems and has serious negative effects on the global environment. This paper presents a new efficient ballast water analysis and heat treatment system using waste heat recovery system on ships. The project demonstrates laboratory methods to verify killing efficiency of micro-organisms in sea water exposed to heat treatment over a short period of time. Heating times were varied in a range from 20 seconds to 3 minutes. The micro-organisms were measured using a flow cytometry instrument and fluorescence microscopy to detect living and dead organisms in untreated and treated water. Based on the biological analysis, a related heat treatment simulation was carried out to confirm the control method.

1. INTRODCUTION

Water has been used as ballast to stabilise vessels at sea for over a hundred years. Ballast water is pumped inside vessels to maintain the correct operating conditions throughout a voyage. This is essential for safe and efficient modern shipping operations. This practice reduces stress on the hull, provides transverse stability, improves propulsion and manoeuvrability, and compensates for weight loss due to fuel and water consumption. Shipping moves over 80% of the world's commodities and transfers approximately three to five billion tonnes of ballast water internationally each year. Ballast water is essential to the safe and efficient operation of modern shipping, providing balance and stability to unladen ships [1].

Ballast water discharged by ships can have a negative impact on the marine environment. Cruise ships, large tankers, and bulk cargo carriers use a large amount of ballast water, which is often pumped-in from one region's coastal waters after waste-water has been

discharged or cargo has been unloaded. The ballast water is then discharged at the next port, or wherever more cargo is loaded. Ballast water discharge typically contains a variety of biological materials, including plants, animals, viruses and bacteria. These materials often include non-native, exotic species, which can cause extensive ecological and economic damage to aquatic ecosystems. Therefore, ballast water poses serious ecological, economic and health problems. Transferred species may survive and establish a reproductive population in the host environment, becoming invasive, out-competing native species and multiplying into pest proportions.

The purpose of this project is to develop methods for verification of treatment methods to be used in designing new ballast water handling systems at Aalesund University College. Our research is all based on the International Maritime Organization (IMO) Ballast Water Management Convention 2004, which will come into force in 2013 / 2014 after it has been ratified by nations representing more than 35% of the world's Gross Tonnage.

Both Flow cytometry and fluorescence microscopy were used and proven to be efficient instruments in the verification process. Furthermore, the biological tests indicated that the high temperature (80 °C) might be required in order to ensure efficient killing of micro-organisms (under ten microns) within 60 seconds of heating time. The larger organisms (phytoplankton and zoo-plankton) are almost killed at lower temperatures. After that, the water treatment process system was modelled using Bond Graph and 20SIM software. The objectives of the process simulation model were first of all to perform design optimisation on the different components in the system and second, to further simulate the dynamics in order to implement a proper control loop. At last, the conclusions and future work are given.

2. RELATED WORK

Scientists first recognised the signs and effects of the introduction of an alien species after a mass introduction

of the Asian phytoplankton algae *Odontella* (*Biddulphia sinensis*) in the North Sea in 1903. In the late 1980s, Canada and Australia were among countries experiencing particular problems with invasive species, and they brought their concerns to the attention of the International Maritime Organization's Marine Environment Protection Committee (MEPC) [2]. The problem of invasive species in ships' ballast water is now worsening. This is largely due to the expanded trade and traffic volume over the last few decades and since the volumes of seaborne trade continue to increase, the problem may not have reached its peak yet.

The last few decades have seen considerable progress in research on ballast water treatment and management. There is an urgent need in the shipping industry for the development of cost-effective and environmentally friendly Ballast Water Management Systems (BWMSs). According to the Ballast Water Convention, the International Maritime Organization (IMO) has set the Ballast Water Exchange Standard, D1 and the Ballast Water Performance Standard (BWPS), D2 (Table 1). The IMO Convention sets discharge limits on densities of live organisms based on the organism's size class. Organisms with a size that is greater than or equal to 50 μm mostly represent zoo-plankton, and organisms that are at least 10 μm but are smaller than 50 μm are mostly comprised of phytoplankton. Using zoo-plankton and phytoplankton as categorisation groups allows for a broad and important comparison of the results obtained in relation to the new standard [3, 4].

Table 1. The IMO ballast water performance standards (D-2)

Organism category	Standard
Organism size $\geq 50 \mu\text{m}$	< 10 viable organisms/mL
$10 \mu\text{m} \leq$ Organism size < $50 \mu\text{m}$	< 10 viable organisms /mL
Organism size < $10 \mu\text{m}$ (including the following items)	
Toxicogenic <i>Vibrio cholerae</i>	< 1 cfu/100 mL
<i>Escherichia coli</i>	< 250 cfu/100 mL
Intestinal Enterococci	< 100 cfu/100 mL

Many technologies have been under development during the negotiations at IMO, but it is difficult to compare the efficiency of treatments at removing organisms as, until the convention was adopted, there were no set standards.

The heat treatment of ballast water has been widely advocated as a possible treatment regime based on theoretical and laboratory/small scale trials. Various methods of heating the ballast water on board vessels have been previously used. The length of time the water was heated varied from 20 h at temperatures in excess of 35 $^{\circ}\text{C}$, 15 h at 42 $^{\circ}\text{C}$ and 80 h at more than 30 $^{\circ}\text{C}$ [5, 7].

Previous experiments carried out on board vessels have achieved a 90 – 100% reduction of the phytoplankton and zoo-plankton by using waste engine heat to treat the ballast at 35 – 38 $^{\circ}\text{C}$ for 20 h [6] and a 100% kill rate of zoo-plankton by heating the ballast water to 38 $^{\circ}\text{C}$ for 12 h [5, 7]. Instant exposures at high temperatures (40 – 65 $^{\circ}\text{C}$) have already been tested in the laboratory with successful results for phytoplankton and zoo-plankton [8, 9, 10].

This pre-project dealt with the application of this high temperature treatment under operational conditions. The aim was to assess the extent to which this method was able to treat the organisms smaller than 50 μm (phytoplankton and bacteria) in the ballast water.

3. TEST METHODS AND EQUIPMENT

3.1 Test equipment

Freshly collected sea water from "Åsefjord" containing natural strains of phytoplankton and the culture laboratory strain of *Escherichia coli* was prepared. The tests were performed at 51.8 $^{\circ}\text{C}$, 74 $^{\circ}\text{C}$, and 81 $^{\circ}\text{C}$ water bath for holding time of 10, 20, 30, 40, 50, 60, 90, 120 and 180 seconds. After treatment, the heated test tubes were directly removed from hot bath and placed into an ice bath to cool down for analysis. 100 $^{\circ}\text{C}$ treatment was used as a positive control. Test samples before and after treatment were then compared.

The viability of phytoplankton was measured by staining with SYTOX Green and carrying out tests involving flow cytometry assay and fluorescence microscopy. The viability of E-coli cells was measured by staining with SYBR Green, SYTO 9, or SYTOX Green, together with propidium iodide (PI), and tested by flow cytometry assay and fluorescence microscopy. Temperature of the water bath (Heto, Birkerød Denmark) at different levels: 22 $^{\circ}\text{C}$, 37 $^{\circ}\text{C}$, 35.5 $^{\circ}\text{C}$, 51.8 $^{\circ}\text{C}$, 69 $^{\circ}\text{C}$, 74 $^{\circ}\text{C}$ and 81 $^{\circ}\text{C}$.

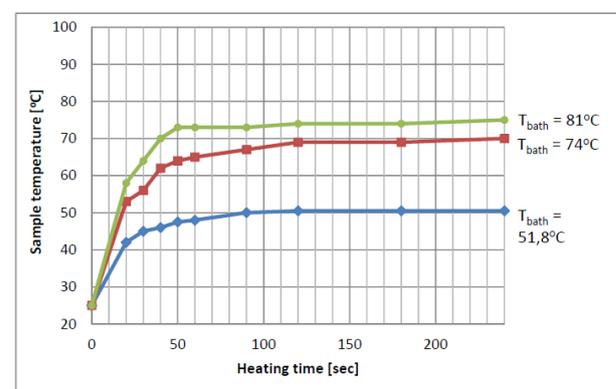


Figure 1: Temperature of 4 ml of water in Kimax glass tubes (10ml) in water bath at 51.8 $^{\circ}\text{C}$, 74 $^{\circ}\text{C}$ and 81 $^{\circ}\text{C}$.

Different test tubes were tried in the evaluation of heating efficiency by measuring temperature rise during heating in the water bath. These tubes and conditions

are: Nunc polypropylene tube (2 ml) with 2 ml of water, Kimax glass test tubes with screw caps (10 ml) with 10 ml of water, and Conical polypropylene centrifuge Tubes (15 ml) with 15 ml of water.

Results in our tests clearly show that Kimax glass test tubes provide the most efficient heating. As such, these are chosen for the further experiments. Temperature control in water bath was tested using 4 ml of water in 10 ml Kimax glass tubes measuring temperature rise in sample water. Results (Figure 1) show that the maximum temperature is reached after approximately 1 minute.

3.2 Organisms of different sizes

3.2.1 Organisms of size range between 10-50 μm

Organisms whose size are greater than or equal to 50 μm mostly represent zoo-plankton, and organisms whose size is less than 50 μm but more than 10 μm are mostly comprised of phytoplankton. Using zoo-plankton and phytoplankton as categorisation groups allows for a broad and important comparison of the results obtained in relation to the new standard.

Organism viability is not easily detected by a single morphological, physiological, or genetic parameter, making it advantageous to use more than one approach. Even procedures recommended in the protocol for land-based verification testing have practical limitations because of time constraints. There are no standard methods for readily and reliably discerning live and dead organisms of the 10-50 μm group. Flow cytometry can provide a rapid automated assessment of phytoplankton assemblages, especially since several recent advances have mitigated mechanical problems, like clogging, that have hampered its use in phytoplankton research in the past.

3.2.3 Micro Organisms small than 10 μm

Escherichia coli (*E. coli*) are 2-3 μm long rod-shaped bacteria, with a selectively permeable cell membrane and DNA held in a nucleoid area. Current best practice detection techniques for the viability of *E. coli* are based upon cell culture requiring 18 to 24 hours for a result.

Hopefully the device will be able to produce a quantitative result in less than an hour. Flow cytometry is rapid, easy, and sensitive for live/dead bacteria counting. Knowledge of the living/non-living and active/inactive states of cell populations is fundamental in understanding the role and importance of micro-organisms in natural ecosystems. Many approaches are based on membrane integrity, such as the Live/Dead kits (e.g. the LIVE/DEAD BacLight bacterial viability kit from Molecular Probes), a propidium iodide (PI) based assessment of dead cells. Usually, a combination of SYBR Green dyes or SYTO 9 and PI is widely employed for analysing live/dead bacteria numbers.

3.2.3 Calibration of flow cytometry size

The Flow Cytometry Size Calibration Kit has non-fluorescent particle-size calibration standards that provide a simple, accurate way to determine cell sizes by flow cytometry. The kit contains six suspensions of highly uniform polystyrene micro-spheres with the following diameters: 1.0 μm , 2.0 μm , 4.0 μm , 6.0 μm , 10.0 μm and 15.0 μm . The size of the plankton and bacteria were determined by comparison to standardised beads (10 and 50 μm).

4. WATER HEATING TREATMENT RESULTS

4.1 Water samples heated in a 74 °C water bath (flow cytometry results)

Fluorescence of SYTOX Green, a dye that only penetrates damaged cell membranes, and autofluorescence were observed simultaneously, allowing the discrimination of live and dead cells [12]. Freshly collected sea water samples were pre-filtered using a sieve made of a HYDRO-BIOS 50 μm mesh net. The 50 μm pre-filtered samples were for further treatment and analysis.

Before and after exposure to different length of heating, the 50 μm pre-filtered sea water samples were stained with SYBR and analysed on a flow cytometer (Figure 2). As shown in Figure 3, Natural red autofluorescence (red, FL-3H) from chlorophyll could identify viable phytoplankton cells (P1). Bright green fluorescence was observed in the samples that had been stained with SYTOX Green. These samples contained heated cells. After 20 seconds of treatment in a 74 °C water bath, 96% of the phytoplankton was killed (P2). After being heated for 180 seconds, no viable cells (P1) were detectable.

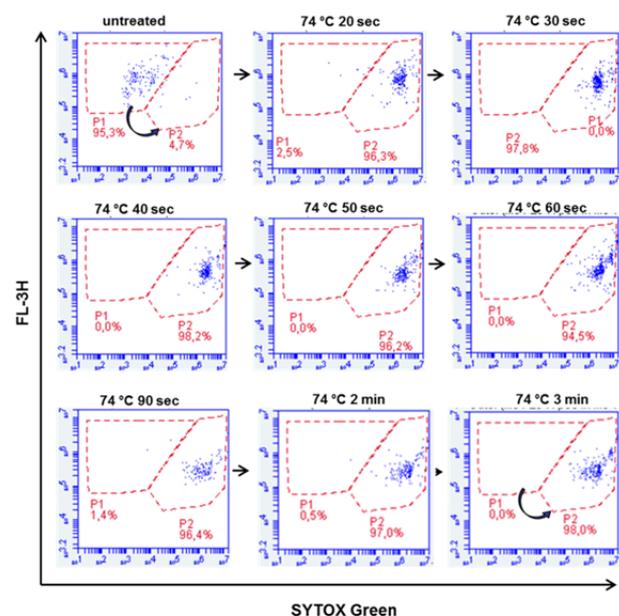


Figure 2: Flow cytometry results of 74 °C treated samples. P1: viable cells, P2: dead cells.

4.2 Comparison of 74 °C and 51.8 °C water bath

The 50 µm pre-filtered sea water samples were treated in either a 74 °C or a 51.8 °C water bath, then stained with SYBR and analysed on a flow cytometer, as shown in Figure 3. After three minutes of heating in water bath, the 51.8 °C treated group had still 1.2 % viable cells, while none were present in the 74 °C treated group.

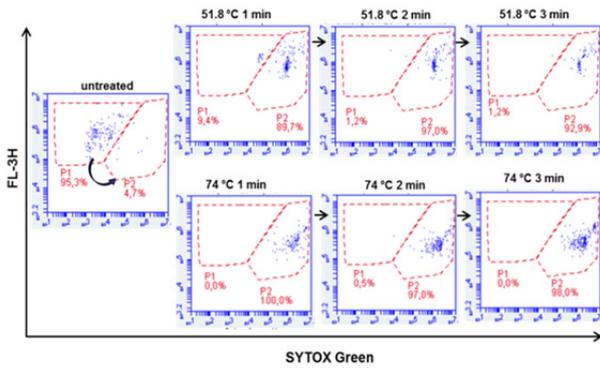


Figure 3: Treatment efficiency of a 74 °C and a 51.8 °C water bath. P1: viable cells; P2: dead cells.

4.3 Fluorescence microscopy test

Figure 4 shows that natural red autofluorescence from chlorophyll could identify viable phytoplankton cells (upper). Bright green fluorescence was observed in the samples that had been stained with SYTOX Green. These samples contained heated cells (lower). In the figure, the red part represents viable cells, and green part represents dead cells.

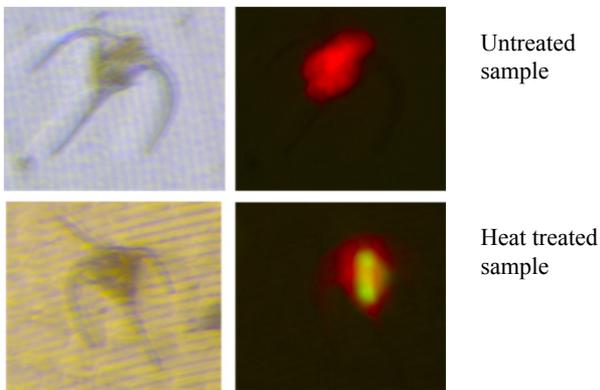


Figure 4: Natural red autofluorescence from chlorophyll.

4.4 E. coli heated in 80°C water bath

Flow cytometric analyses of E. coli before and after being heated are shown in Figure 5.

Before and after exposure to different amounts of heating, bacterial cell samples were stained either with a mixture of SYBR Green plus PI or with only SYBR Green and analysed on a flow cytometer. After 20 seconds of treatment in an 80 °C water bath, staining with SYBR Green and PI showed intermediate states.

After being heated for 90 seconds, all cells were PI positive. The green colour show viable cells, while the red indicates dead cells.

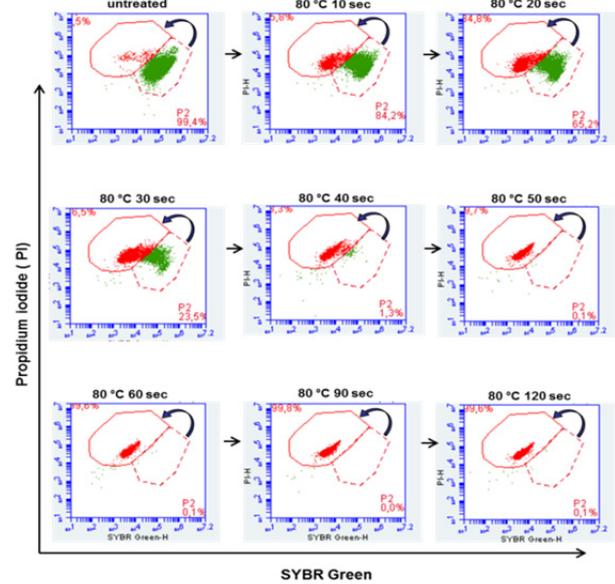


Figure 5: Flow cytometric analysis of E. coli before and after heat treatment. Green: viable cells, Red: dead cells.

4.5 Three methods for E. coli viability detection

Flow cytometric analysis of E. coli. Bacterial cells were heated at 100 °C for one minute or in an 80 °C water bath for two minutes. Untreated and treated bacterial cell samples were stained with a mixture of either SYBR Green (left), SYTO9 (middle) or SYTOX Green (right) plus PI and analysed on a flow cytometer. Results are plotted in Figure 6, where P1 (red) are dead cells P2 (green) are viable cells.

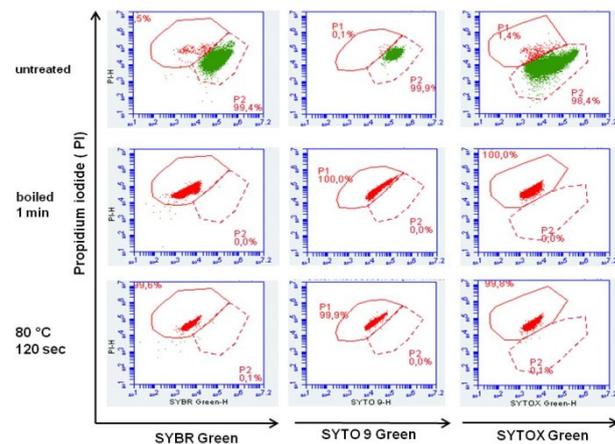


Figure 6: Flow cytometric analysis of E. coli bacterial cells heated at 100 °C for one minute or in 80 °C for two minutes. P1(red): dead cells P2 (green): viable cells.

Further fluorescence microscopy analysis of E. coli was performed on the same samples. Results are shown in Figure 7. This project has demonstrated laboratory methods to verify killing efficiency of micro-organisms

in sea water exposed to short time heat treatment. Heating times were varied in the range from twenty seconds to three minutes. Flow cytometry assay and fluorescence microscopy are used to measure living and dead organisms in untreated and treated water.

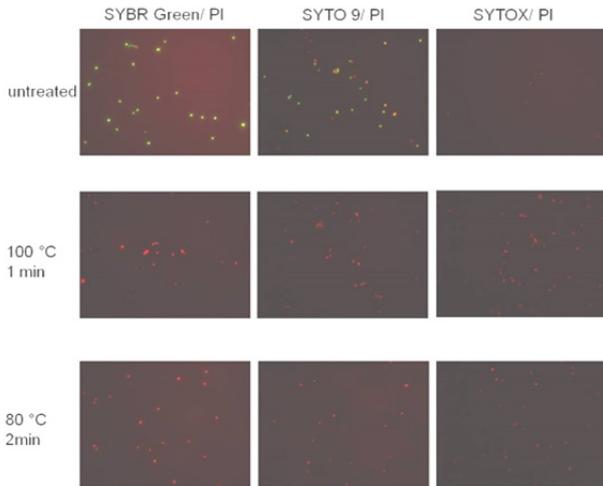


Figure 7: Fluorescence microscopy analysis of E. coli.

5. BALLAST WATER TREATMENT SIMULATIONS

In this part of the project we will develop simulation models for a ballast water treatment system that employs a waste heat recovery system. The simulator should allow for the optimisation of heat use by way of an efficient combination of heat exchangers, reactors and a control system. The aim is to assess the extent to which this method is able to treat the organisms under $50 \mu\text{m}$ (phytoplankton and bacteria) in ballast water within a heating time of one minute.

The schematic of the water treatment process system is shown in figure 8. The main components of the system are the *heat recovery unit* and the *reactor* where bio-treatment follows a temperature-time history.

The system is modelled using Bond Graph method [13, 14, 15] and 20SIM software [16]. The overall system model is shown in Figure 9 and the heat recovery unit is shown in Figure 10. The Bond Graph is a unified

modelling tool for multi domain systems where energy flow (power) and preservation of energy and mass are the common variables. The basic model consists of energy storing elements (C-elements and I-elements) and energy transferring or dissipative elements (R-elements). In figure 10 the R-elements represent the convective and conductive heat flows while the C-elements are the heat capacitance in each model segment. The I-elements represent the hydraulic inertia in the fluid flow. An icon-based object-oriented modelling interface is provided by 20SIM, where the overall system layer is shown in Figure 9.

The objectives of the process simulation model are to optimally design the different components in the system and to further simulate the dynamics in order to implement a proper control loop. The purpose of the treatment system is to heat and keep the temperature at $T_{\text{reactor}} = 80 \text{ }^\circ\text{C}$ for 30 seconds. The water is then cooled down in the recovery unit in order to save energy. The objective is to minimise the need for boost heating in the super-heater.

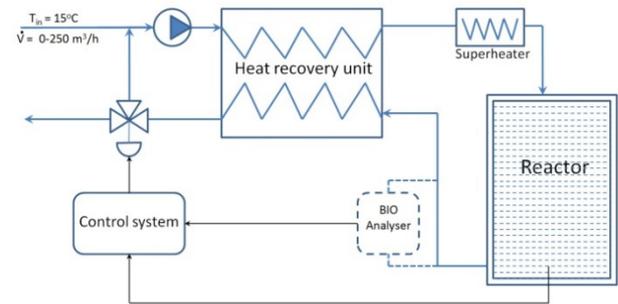


Figure 8: Water treatment system.

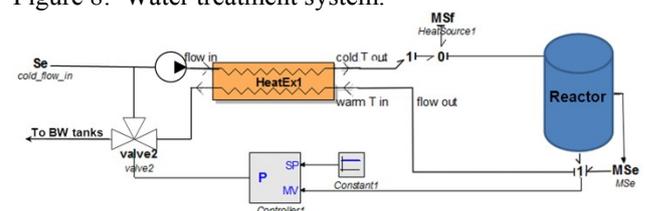


Figure 9: Treatment system simulation model.

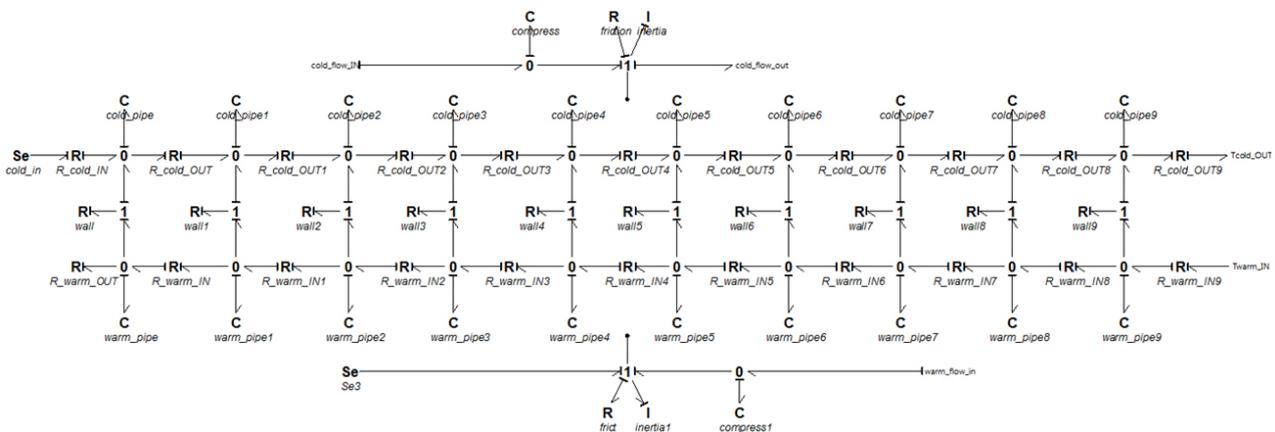


Figure 10: Heat recovery unit model.

In the first part of the simulation study, the initial heating and cooling process was simulated. The results showed that the system reached the required temperatures and initial heating time as required. Figure 11 shows the temperature-time history for the reactor and inlet/outlet temperatures for the heat recovery unit simulate a start and stop cycle. Further simulations included a control loop to secure optimal flow and power control for a continuous process. Figure 12 shows the temperature history for the water flowing through the continuous process simulating different flow control. The next steps will be to implement a Bio-analyser and to model the bio-reactor function.

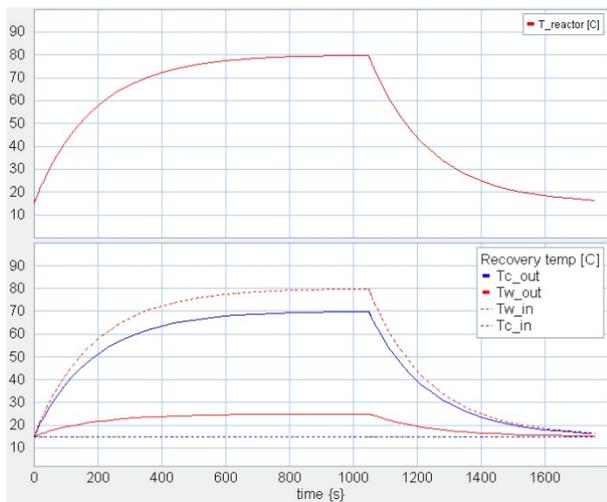


Figure 11: Simulated heating and cooling process.

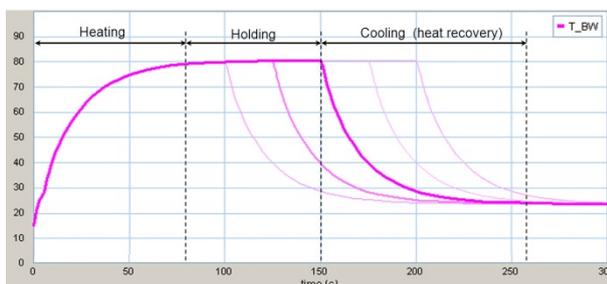


Figure 12: Water temperature history simulation using different flow control parameters.

6. CONCLUSIONS AND FUTURE WORK

Ballast water treatment is still a very challenging area. Many technologies have been under development during negotiations at IMO, but it has been difficult to compare the efficiency of treatments at removing organisms as, until the convention was adopted, there were no set standards. The first step in our project is to investigate the current ballast water treatment situation, and to focus on the methods. Therefore, more extended tests are needed to evaluate the efficiency of the treatment method.

From a biological analysis viewpoint, both flow

cytometry and fluorescence microscopy have proven to be efficient instruments in the verification process. The results from the preliminary experiments indicated that heat treatment is efficient in reducing the viable phytoplankton in natural sea water and laboratory cultures of *Escherichia coli*. The killing efficiency depends on the heating temperature and the holding time. This result will be further verified using the classic membrane filtration method in the future work.

Furthermore, the preliminary results indicated that a high temperature (80 °C) is probably required for the bacteria under 10 microns in size (such as *E. coli*), in order to guarantee efficient killing within 60 seconds of heating time. The larger organisms (phytoplankton and zoo-plankton) are killed at lower temperatures.

The ballast water treatment simulation verifies the possibility of using waste heat recovery systems on board ships to deal with the problem. The final goal of the project is to develop methods and equipment for verification and energy optimisation in design of new ballast water treatment and management systems.

The proposed project is still the first step of a long-term project. We will have the following work including developing biological methods for verification of ballast water treatment, developing a ballast water management simulator using waste heat recovery, and proposing the treatment machinery system for real applications.

ACKNOWLEDGMENTS

The project is supported by a VRI-project in Norway. The research work is based on the close cooperation with ULMATEC – Pyro. The authors would like to thank for the contribution from Mr. Yue Li for his support on the 20-sim modelling.

REFERENCES

- [1] <http://globallast.imo.org/index.asp?page=problem.htm&menu=true>
- [2] <http://www.imo.org/OurWork/Environment/BallastWaterManagement/Pages/Default.aspx>
- [3] Anonymous, Guidelines for approval of ballast water management systems (G8). Annex3 Resolution MEPC.125(53), Annex, Parts 1,2,3 and 4., 2005.
- [4] PhD, M.J.W.V., Final report of the land based testing of the BalPure® BWT System. Royal Netherlands Institute for Sea Research, 2009.
- [5] Quilez-Badia, G., et al., On board short-time high temperature heat treatment of ballast water: a field trial under operational conditions. *Mar Pollut Bull*, 2008. 56(1): p. 127-35.
- [6] G. Rigby, G.M.H., C. Sutton, Novel ballast water heating technique offers cost-effective treatment to reduce the risk of global transport of harmful marine organisms. *Marine Ecology Progress Series*, 1999. 191: p. 289-293.
- [7] Mountfort, D.O., Dodgshun, T., Taylor, M., , Ballast Water Treatment by Heat - New Zealand Laboratory and Shipboard trials. In: 1st International Ballast Water Treatment R&D Symposium, 26-27 March, 2001, No. 5.

IMO, London, pp. 45-50. 2001.

- [8] McCollin, T.A., Shanks, A., Biological Assessment. Phytoplankton Results. DTR-3.7.2-FRS-06.03. MARTOB - On Board Treatment of Ballast Water (Technologies Development and Applications) and Application of Lowsulphur Marine Fuel, Newcastle upon Tyne, UK, 27th June, 2003, 87pp. 2003.
- [9] Quílez-Badia, G., Gill, M.E., Frid, C.L.J., Biological Assessment. Zooplankton Results. DTR-3.7.1-UNE-08.03. MARTOB - On Board Treatment of Ballast Water (Technologies Development and Applications) and Application of Lowsulphur Marine Fuel, 29th August 2003, Newcastle upon Tyne, UK, 39pp. 2003.
- [10] Euan D. Reavie, A.A.C., and Lisa E. Allinger Assessing Ballast Water Treatments: Evaluation of Viability Methods for Ambient Freshwater Microplankton Assemblages. *Journal of Great Lakes Research* 2010. 36(3): p. 540-547.
- [11] Berney, M., H.U. Weilenmann, and T. Egli, Flow-cytometric study of vital cellular functions in *Escherichia coli* during solar disinfection (SODIS). *Microbiology*, 2006. 152(Pt 6): pp. 1719-29.
- [12] Masanori Sato, Y.M., Mika Mizusawa, Hitoshi Iwahashi, and Shu-ichi Oka, A Simple and Rapid Dual-fluorescence Viability Assay for Microalgae. *Microbiology and Culture Collections*, 2004. 20(2): p. 1342-4041.
- [13] Pedersen, E., Modelling multicomponent two-phase thermodynamic systems using pseudo-bond graphs, 2001 International Conference on Bond Graph Modelling and Simulation (ICBGM'01); Nov. 2001.
- [14] Karnopp, D.C., Margolis, D.L., & Rosenberg, R.C., *System Dynamics: A Unified Approach*. John Wiley & Sons, Inc., second edition, 1990.
- [15] Thoma, J.U., & Richter, D.B., Simulation of Fluid Pipes in Hydrostatic Circuits Using Modal and Segmented Methods. *Transactions of The Society of Computer Simulation*, Vol. 3, (no. 4):pp.337-349, October 1986.
- [16] <http://www.20sim.com>

AUTHOR BIOGRAPHIES

Yanran Cao received her M.D. degree in 2004 from the Chinese Academy of Medical Sciences & Peking Union Medical College (CAMS & PUMC) in China. From 2004 to 2011, she worked as a post-doctoral research fellow in one group of tumour immunology, Department of Oncology/Haematology, University Medical Centre Hamburg-Eppendorf (UKE). In 2013, she received her Ph.D in Biology, Department of Biology, University of Hamburg. Since January 2012, she has worked as a researcher at the Faculty of Life Science at Aalesund University College.

Vilmar Æsøy received his Ph.D. in Mechanical Engineering in 1996 from Norwegian University of Science and Technology. From 1997 to 2002 he worked as a researcher in Aker Maritime and R&D manager in Rolls-Royce Marine As. Since 2002 he has been working as an associate professor at Aalesund University College.

Anne Stene received her Dr. Scient. in 1998 as judged by a selection committee. In 2013, she received a Ph.D in epidemiology from the Norwegian School of Veterinary Science. Since 1998 she has worked with education, research, administration and consultancy at Aalesund University College regarding marine ecology, fishery and aquaculture. She has also worked in the Norwegian Directorate of Fishery and in the Norwegian Ministry of Fishery and Coastal affairs.