The Activity of Microalgae in Ballast Water based on Microfluidic Chip Electrokinetic Technology

Runzhe Sun[1][a], Zhen Li[2][b], Zhen Liu[3][c], Na Li[3][d] and Yongxin Song[3][e]
[1]*China Classification Society Tianjin Office, Tianjin 300457, China*
[2]*CRRC Changchun Railway Vehicles CO., LTD, Changchun 130000, China*
[3]*Department of Marine Engineering, Dalian Maritime University, Dalian 116026, China*

Keywords: Ballast Water, Detection of Microalgae Activity, Contour Detection, Optical Flow.

Abstract: To control the marine bio-invasions in ballast water, the regulatory discharge standards specify the number of viable organisms in ballast water treatment. It is an important task to determine the activity of microalgae after ballast water treatment. In the current study, five kinds of microalgae were detected on microfluidic chip. The electrokinetic velocity (EV) and diameter of microalgae were measured manually using an optical microscope. Finally, the contour detection and Lucas-Kanada (L-K) Optical Flow technique were used to calculate the diameter and velocity of microalgae, respectively. The result found that the EV of different species of living microalgae decreases with increasing diameter. The EV of dead *Pyramimonas sp.*, *Platymonas* and *Prorocentrum donghaiense* decreased to 0 μm/s. In addition, the L-K optical flow technique can obtain the movement velocity of microalgae at any time, which can improve the detection accuracy. Those study demonstrate that the development of a new field ballast water analysis instrument based on contour detection and L-K optical flow technique is of great significance.

1 INTRODUCTION

During the daily operation, the ships have a large number of ballast water in addition to transporting a variety of cargo. However, the ballast water contains large amounts of biological communities and pathogens, which are the main sources of invasive species in freshwater and marine ecosystems (Sieracki et al., 2014). According to statistics, 3,000 species migrate with ballast water every day. The foreign biological invasion will not only destroy the biodiversity and ecological environment of the original waters, but also have a serious negative impact on the utilization of Marine resources and Marine economy worldwide (Lymperopoulou and Dobbs, 2017). In addition, the biological characteristics of many microorganisms can promote intrusion in ballast water. Because they have a high ability to reproduce asexually and form dormancy stages, which will increase the chance of successful invasion(Ruiz et al., 2000). The microalgae were the main phytoplankton to be detected in ballast water. Therefore, detection of microalgae activity after ballast water treatment is an important part of the inspection process. Currently, the main technology to detect algae activity include flow cytometry, chlorophyll fluorescence and cell staining (Song et al., 2021).

Flow cytometry is an instrument for analysing cell parameters based on optical principles. The main principle is to disperse the samples to be detected into suspension and dye with fluorescent reagent. Under the irradiation of excitation light source, the living cells will emit fluorescence, and the photodetector can judge the activity of microalgae by detecting the fluorescence intensity of the cells (Joachimsthal et al., 2004). Compared with epifluorescence direct counting, flow cytometry has a higher degree of automation (Joachimsthal et al., 2003). However, flow cytometry has limited accuracy in detecting low concentrations of cells. The technicians required to have certain technical capacity, which led to some limitations of flow cytometry in the detection of ship ballast water.

[a] https://orcid.org/0000-0002-7696-1962
[b] https://orcid.org/0000-0002-8071-8068
[c] https://orcid.org/0000-0002-2504-7062
[d] https://orcid.org/0000-0002-0621-2720
[e] https://orcid.org/0000-0001-9877-4335

Sun, R., Li, Z., Liu, Z., Li, N. and Song, Y.
The Activity of Microalgae in Ballast Water based on Microfluidic Chip Electrokinetic Technology.
DOI: 10.5220/0011201100003443
In *Proceedings of the 4th International Conference on Biomedical Engineering and Bioinformatics (ICBEB 2022)*, pages 283-287
ISBN: 978-989-758-595-1

Microalgae contain chlorophyll for photosynthesis (Li et al., 2021). When the chlorophyll is irradiated by an external laser, its internal energy is in an unstable state. During the transition from the ground state to excited state, chlorophyll absorbs the energy brought by the external laser. Eventually, the electrons return to their ground state and the excess energy is released outward as fluorescence. Therefore, the activity of microalgae can be characterized by measuring the intensity of fluorescence. However, some microalgae do not have chlorophyll, such as microalgae living in the deep sea, cyanobacteria and heterotrophs, which cannot be detected by chlorophyll fluorescence technology (Steinberg et al., 2011).

The cell staining can be divided into non-fluorescent staining and fluorescent staining. Neutral red and Trypan blue are commonly used in non-fluorescent staining. Neutral red or Trypan blue dyes can only stain living or dead algae, respectively (Bradie et al., 2017; Stehouwer et al., 2013). Fluorescence staining with fluorescein diacetate (FDA) is a popular method for phytoplankton vitality assessment. However, FDA could not stain all living microalgae, which underestimated the true number of viable microalgae (Hyun et al., 2018). In addition, this method can only estimate the number of microalgae by the total fluorescence intensity, rather than accurately calculate the number of microalgae (Song et al., 2021).

As mentioned above, all three methods have certain limitations. Therefore, it is still necessary to develop new technologies for microalgae activity detection. For most microalgae, the negative charge on cell surface is due to the presence of carboxyl, amino, hydroxyl and phosphate anionic groups (Keller et al., 2015). The surface charge and Zeta potential of microalgae changed with the species and growth process (Ives, 1959). In this study, we first inactivated the algae with sodium hypochlorite. Then, the electrokinetic velocity (EV) of live and dead microalgae in ballast water was measured. Meanwhile, image processing methods such as edge detection and Lucas-Kanada (L-K) Optical Flow technique are used to optimize the measured parameters in the process of electric motion. A method of microalgae activity detection based on flow velocity is proposed, and the core objective is to provide a method basis for ballast water compliance.

2 MANUSCRIPT PREPARATION

2.1 Preparation of Microalgae

Chlorella vulgaris (*C. vulgaris*), *Dunaliella salina* (*D. salina*), *Pyramimonas sp.*, *Platymonas* and *Prorocentrum donghaiense* (*P. donghaiense*) were used in the experiments. The experiments need living and death of algae. So, we inactivated microalgae by treating them with 10 mg/L sodium hypochlorite for 5min. Then, the method of neutral red was used to stain microalgae in vivo to verify the cell activity (Olsen et al., 2015). According to the staining results, microalgae have been inactivated after sodium hypochlorite treatments (Figure. 1). To enable microalgae to be added to the microfluidic chip, the living or dead algae were centrifuged for 3 min at 4000 rpm with a centrifuge (Eppendorf 5424, GER). After abandoning the supernatant, 10% PEG was added into the 1.5 mL centrifuge tube and centrifuged again. PEG-living microalgae mixture and PEG-dead microalgae mixture were obtained respectively.

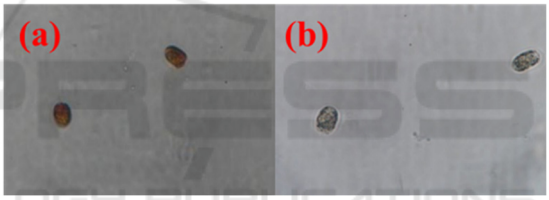

Figure 1: Illustration of neutral red staining for *Pyramimonas sp.* (a) living algae and (b) dead algae.

2.2 Microchannel System

The microchannel (1 cm×100 μm×25 μm, length × width × height) and the slide coated with PDMS were immersed in 10% PEG solution for 10 min. Afterwards, the excess solution on the microfluidic chip was dried and at 80 °C for more than 10 h in the drying oven. Finally, the modified microfluidic chip was obtained (Song et al., 2021) (Figure. 2).

The positive and negative platinum electrodes are placed at the exit and entrance of the microchannel, respectively. Add 10 μL PEG-microalgae mixture and 10 μL 10% PEG solution to the inlet and outlet of the channel, respectively. Meanwhile, adjust the liquid level at both ends of the channel and apply an electric field of 50 V/cm after keeping the microalgae stationary. The movement distance of microalgae was recorded under the inverted optical microscope imaging system (TI-E, Nikon, Japan). The diameter and EV

of microalgae were calculated by manual and algorithm respectively.

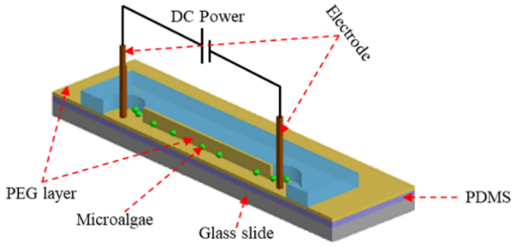

Figure 2: Schematics of microfluidic chip.

2.3 Second Section

The target microalgae were extracted by background subtraction method to eliminate the influence of background. Then, we used the OTSU method to binarize the extracted target microalgae to reduce the internal texture. Finally, Canny operator is used to detect its edge. To remove the contour error caused by texture, the erode function in OpenCV is used to corrode the image. The L-K optical flow technique based on image pyramid is realized by calcopticFlowpyrLK function, and the optical flow of corresponding corner points is predicted.

3 RESULT AND DISCUSSION

3.1 Manual Measurement of Electrokinetic Velocity and Diameter in Algae

The relationship between EV and diameter of living microalgae in this experiment is shown in Figure. 3. The EV of different species of microalgae decreases with increasing diameter. For instance, the EV of *C. vulgaris* is found from 21.13 μm/s to 18.83 μm/s when the diameter ranges from 3.23 μm to 4.21 μm. In general, the EV is related to the number of anionic groups on the cell surface, gravity effect and friction effect on the channel wall. Therefore, the different sizes of microalgae are affected to different degrees, resulting in differences in EV. According to the dead microalgae, the average EV of *C. vulgaris*, *D. salina*, *Pyramimonas sp.*, *Platymonas* and *P. donghaiense* decreased to 2.79 μm/s, 2.13 μm/s, 0 μm/s, 0 μm/s, and 0 μm/s with the inactivation, respectively (Figure. 3).

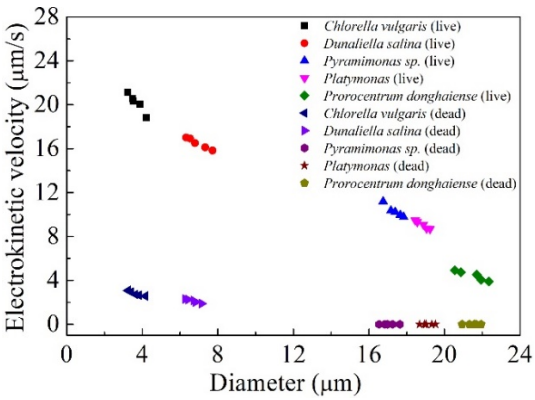

Figure 3: The relationship between algae diameter and EV measured by manual measurements.

The main reason for the decrease of velocity may be that the stop of algae metabolism and the passivation of surface anionic groups lead to the decrease of Zeta potential.

3.2 Contour Detection

The contour detection method was used to measure the size of 50 randomly selected microalgae samples. The results were compared with the manual measurement, as shown in Figure. 4. The size of *C. vulgaris*, *D. salina*, *Pyramimonas sp.*, *Platymonas* and *P. donghaiense* differed by 0.35 μm, 0.41 μm, 0.84 μm, 0.27 μm and 0.62 μm from that measured manual measurement, respectively. It was found that manual measurement had a better effect on large microalgae (*Pyramimonas sp.*, *Platymonas* and *P. donghaiense*), while contour detection was more advantageous for small microalgae (*C. vulgaris* and *D. salina*). Therefore, the technology of contour detection can not only improve the detection accuracy of diameter parameters in algae, but also reduce the work of experiments.

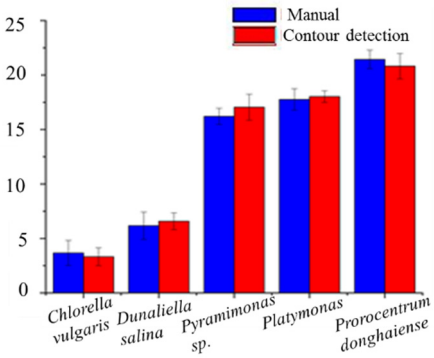

Figure 4: Detection results of algae by contour detection and manual measurements.

3.3 Lucas-Kanada Optical Flow Technique

As shown in Table 1, the EV of living microalgae was measured by L-K Optical Flow technique. The same species of microalgae had similar EV at different locations in the culture medium. The average EV of *C. vulgaris*, *D. salina*, *Pyramimonas sp.*, *Platymonas* and *Prorocentrum donghaiense* was 21.81 μm/s, 15.36 μm/s, 10.24 μm/s, 8.45 μm/s and 4.77 μm/s, respectively. In addition, we also measured the EV of dead microalgae, in which we found that the average EV of *Pyramimonas sp.*, *Platymonas* and *P. donghaiense* decreased to 0 μm/s (Table 1). For small microalgae, the velocity of C. vulgaris and *D. salina* decreased to 2.84 μm/s and 2.36 μm/s, respectively.

In order to verify the reliability of the L-K Optical Flow technique, the results are compared with the manual measurements. The EV deviation of living *C. vulgaris*, *D. salina*, *Pyramimonas sp.*, *Platymonas* and *P. donghaiense* measured by the above two methods was 0.91 μm/s, 0.96 μm/s, 0.52 μm/s, 0.76 μm/s and 0.42 μm/s, respectively. In addition, the velocity deviation of dead *C. vulgaris* and *D. salina* was 0.24 μm/s and 0.22 μm/s, respectively (Table 1). The main reason for the above deviation is that the measurement result of L-K Optical Flow technique depends on tracking the movement displacement of the marked pixel level corner point between two frames. Then, calculate the instantaneous velocity from the displacement. However, manual measurement may have artificial errors in the process of distance marking and timing. In contrast, the L-K Optical Flow technique can get the speed at any time and more accurate velocity results.

Table 1: EV of algae was measured by L-K optical flow technique and manual measurements.

	C. vulgaris		*D. salina*		*Pyramimonas sp.*		*Platymonas*		*P. donghaiense*	
	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead
L-K Optical Flow	21.81	2.84	15.36	2.36	10.24	0	8.45	0	4.77	0
Manual	20.90	2.60	16.32	2.14	10.76	0	9.21	0	4.35	0
Deviation	0.91	0.24	0.96	0.22	0.52	0	0	0	0	0

4 CONCLUSIONS

In this study, we propose a method to judge microalgae activity based on EV for evaluating the viability of algae after ballast water treatment. The EV of five different species of microalgae was measured manually by using a microfluidic chip. The result showed that the EV of different species of living microalgae decreases with increasing diameter. The EV of dead large microalgae *(Pyramimonas sp.*, *Platymonas* and *P. donghaiense*) decreased to 0 μm/s, while the small algae of C. vulgaris and *D. salina* decreased to 2.84 μm/s and 2.36 μm/s, respectively. In addition, to avoid time-consuming and susceptible to human factors in the process of manual measurement, the EV parameters of microalgae are optimized by contour detection and L-K Optical Flow technique. It reduces the influence of human factors on EV measurement and improves accuracy.

ACKNOWLEDGEMENTS

This work was supported by the financial support of the National Natural Science Foundation of China (51679023, 51979019) to Y. Song.

REFERENCES

Bradie, J., Gianoli, C., He, J., Lo Curto, A., Stehouwer, P., Veldhuis, M., Welschmeyer, N., Younan, L., Zaake, A., and Bailey, S. (2017). Detection of UV-treatment effects on plankton by rapid analytic tools for ballast water compliance monitoring immediately following treatment. Journal of Sea Research 133, 177-184.

Hyun, B., Cha, H.-G., Lee, N., Yum, S., Baek, S.H., and Shin, K. (2018). Development of an ATP assay for rapid onboard testing to detect living microorganisms in ballast water. Journal of Sea Research 133, 73-80.

Ives, K.J. (1959). The significance of surface electric charge on algae in water purification. Journal of Biochemical & Microbiological Technology & Engineering 1, 37-47.

Joachimsthal, E.L., Ivanov, V., Tay, J.-H., and Tay, S.T.L. (2003). Flow cytometry and conventional enumeration

of microorganisms in ships' ballast water and marine samples. Marine Pollution Bulletin 46, 308-313.
Joachimsthal, E.L., Ivanov, V., Tay, S.T.L., and Tay, J.H. (2004). Bacteriological examination of ballast water in Singapore Harbour by flow cytometry with FISH. Marine Pollution Bulletin 49, 334-343.
Keller, R.P., Drake, J.M., Drew, M.B., and Lodge, D.M. (2015). Linking environmental conditions and ship movements to estimate invasive species transport across the global shipping network. Diversity and Distributions 17, 93-102.
Li, N., Liu, Y., Liang, Z., Lou, Y., Liu, Y., Zhao, X., and Wang, G. (2021). Influence of fuel oil on Platymonas helgolandica: An acute toxicity evaluation to amino acids. Environmental Pollution 271, 116226.
Lymperopoulou, D.S., and Dobbs, F.C. (2017). Bacterial Diversity in Ships' Ballast Water, Ballast-Water Exchange, and Implications for Ship-Mediated Dispersal of Microorganisms. Environmental Science & Technology 51, 1962-1972.
Olsen, R.O., Hess-Erga, O.K., Larsen, A., Thuestad, G., Tobiesen, A., and Hoell, I.A. (2015). Flow cytometric applicability to evaluate UV inactivation of phytoplankton in marine water samples. Marine Pollution Bulletin 96, 279-285.
Ruiz, G.M., Fofonoff, P.W., Carlton, J.T., Wonham, M.J., and Hines, A.H. (2000). Invasion of coastal marine communities in North America: Apparent patterns, processes, and biases. Annual Review of Ecology & Systematics 31, 481-531.
Sieracki, J.L., Bossenbroek, J.M., Lindsay, C.W., and Mckindsey, C.W. (2014). A Spatial Modeling Approach to Predicting the Secondary Spread of Invasive Species Due to Ballast Water Discharge. Plos One 9, e114217.
Song, Y., Li, Z., Feng, A., Zhang, J., Liu, Z., and Li, D. (2021). Electrokinetic detection and separation of living algae in a microfluidic chip: implication for ship's ballast water analysis. Environmental Science and Pollution Research 28, 22853-22863.
Stehouwer, P.P., Liebich, V., and Peperzak, L. (2013). Flow cytometry, microscopy, and DNA analysis as complementary phytoplankton screening methods in ballast water treatment studies. Journal of Applied Phycology 25, 1047-1053.
Steinberg, M.K., Lemieux, E.J., and Drake, L.A. (2011). Determining the viability of marine protists using a combination of vital, fluorescent stains. Marine Biology 158, 1431-1437.