Separation and Purification Technology 169 (2016) 247–252

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur

Inactivation of *Asterionellopsis glacialis* in seawater using combinations of deep ultraviolet light emitting diodes ☆

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ARTICLE INFO

Article history:
Received 14 August 2015
Received in revised form 13 May 2016
Accepted 28 May 2016
Available online 2 June 2016

Keywords:
Disinfection
Algae
Ultraviolet light
Light emitting diodes
Asterionellopsis glacialis
Water treatment

ABSTRACT

Deep ultraviolet light emitting diodes (LED) were studied in water disinfection using *Asterionellopsis glacialis* as a model microorganism. Ultraviolet treatment reactors were constructed and LEDs with peak emission wavelengths 256, 262, 268, 274 and 278 nm and combinations 256 + 262, 262 + 268, 268 + 274 and 274 + 278 were investigated. The photosynthetic activity of the algae was measured using PAM fluorometry and flow cytometry. Dose-based efficiency evaluation with PAM fluorometry did not present any significant distinction between different wavelength LEDs, best performance being with 256 + 262 combination (0.0017 cm^2/mJ). Best performance using flow cytometry analysis was observed with 256 nm LEDs. Best time-based performance with PAM fluorometry was observed using 274 and 278 nm LEDs with inactivation constants of 0.0715 and 0.0680 1/min respectively. The combination 274 + 278 nm performed best according to flow cytometry analysis with inactivation rate constant of 0.1296 1/min. The dose-based efficiency of lower wavelength LEDs can be significantly compensated with higher output power, lower energy consumption and longer lifetime of LEDs with higher wavelengths.

1. Introduction

The microorganisms can be transported from water region to another inside ship ballasts. They can reside for example in the water, sediments or on surfaces in biofilms [6]. Although it is suggested that the biological risks of non-native species transport, caused by ship traffic should be considered and managed as an entirety in place for separated parts and processes [5], ballasts are certainly one of the most important vectors for introduction of non-native species to new sea regions. The International Maritime Organisation (IMO) members have agreed on regulations in ballast water management [9], which amongst others established limits for organism numbers that may be released in specified volumes of water. The regulations, depending on ship ballast capacity and build year, have already entered or will enter into force. The introduction of non-native invasive species can be reduced for example by water exchange in sea regions, although this method does not remove all organisms and is considered effective only in very specific scenarios. Efficiency of ballast water exchange in removing organisms enhanced when the exchange was performed in deep waters and when source port water salinity was low, while the method was not considered effective enough in short regional voyages [14]. Additionally, ballast water exchange can be hazardous during stormy weather [2], thus an application of physical or chemical method is often required.

Technologies for treating ballast water are often derived from municipal and industrial applications, but their usability is limited by space, efficiency and cost. Such alternatives can be separation e.g. filtration or sedimentation. Other alternatives include physical or chemical disinfection. Usage of ultraviolet irradiation is one of the most widely used technology [11]. Ballast water treatment systems using ultraviolet disinfection technology have been developed because of its clear benefits such as minimal by-product formation, broad range and no need to add chemicals [16]. The disruptive effect of ultraviolet light on DNA or RNA [1] is proven to be effective against viruses, bacteria and also protozoans [7,8].

Usage of ultraviolet light emitting diodes (UV-LED) in water treatment has been considered as a very potential alternative to the traditionally used mercury lamps, because they can emit ultraviolet light in chosen optimum narrow waveband and possess few additional benefits, such as compact size, lack of warm-up time and disposal problem [19–23]. Aluminium and gallium nitrides

☆ The studies took place in Lappeenranta University of Technology, Laboratory of Green Chemistry, Mikkeli, Finland. Experiments and analyses were performed by Mikko Rantalankila, Heli Koivistoinen and Lana Sarvasidze, and they were supervised by Professor Mika Sillanpää. This manuscript has not been submitted in any other journal.

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http://dx.doi.org/10.1016/j.seppur.2016.05.045
1383-5866/

are generally used materials in ultraviolet LEDs [4]. LEDs with emission wavelength as low as 210 nm have been constructed using si-doped AlN [17].

Determining the applicability of the LED products on ballast water treatment was the main objective of this study. While there have been publications on ultraviolet treatment efficiency on different algal species [12,13,18], none have demonstrated UVC-LEDs as ultraviolet irradiation source or wavelength dependent determination of algae inactivation efficiency. Since space and energy are limiting factors in ships, LED technology could prove successful in ballast water treatment, and was therefore seen worth a particular investigation. Treatment efficiency was determined from a range of UVC-LEDs by using time- and dose-based evaluation.

2. Materials and methods

2.1. Sample preparation

Two algal species, *Asterionellopsis glacialis* (K-1233) and *Thalassiosira* sp. (K-1314), from Scandinavian Culture Collection of Algae and Protozoa (SCCAP) were used in this study. The species were selected, because they are common in seawater and according to Liebich et al. [10] *Thalassiosira* could be used as indicator due to its relatively high UV tolerance. The *Asterionellopsis* was evaluated as susceptible species in the same article. The species' cell size and optimal growth temperature were also taken into account. The algae were cultivated in synthetic sea water, which was prepared using Milli-Q water, commercial sea salt (Coral Pro Salt, Red Sea) and a stock solution for L1 medium (SCCAP). Salinity of 30.6 PSU and pH of 8.2–8.4 were measured using refractometer (Atago Master) and pH meter (inoLab 730, WTW) respectively. The algae were cultivated under stable conditions in temperature of 25 °C and invariable cycle of 12 h darkness and 12 h of photosynthetically active radiation (daylight) of 80–100 μmol/m^2/s, measured using photoradiometer (HD 2302.0 LightMeter, Delta OHM). The experiments were conducted using algacultures, which had reached late log growth phase on an average of 3 days after inoculation (Fig. 1), observed using PAM fluorometer. The yield was calculated from relation of photosynthetic activity of dark-adapted and light-saturated sample using Eq. (1).

$$Yield = \frac{F_m - F_0}{F_m} = \frac{F_v}{F_m} \quad (1)$$

where

F_m is the maximum fluorescence during saturation pulse,

F_0 is the minimum fluorescence of dark adapted sample.

The initial algal concentration of 1.7–8.6 × 10^4 cells/mL upon treatment experiments was measured using flow cytometry (Becton Dickinson Accuri C6). To reach the limit of IMO ballast water management convention D2 for organisms sized 10–50 μm, a minimum of 3.2–3.9 log inactivation level had to be achieved in the prepared samples.

2.2. Ultraviolet treatment equipment

Two round circuits (Ø 50 mm) of 11 LED components (Sensor Electronic Technology, Inc.) were manufactured for the experiments (LED modules). LED components were placed in two concentric circles (Ø 14 and 37 mm) with 3 LEDs on the inner and 8 on the outer circle. The LED components were installed inside TO-39 semiconductor package with a flat quartz glass window.

LED temperature, input voltage and current were monitored with an external platinum resistance thermometer (Fluke 16) and a multimeter (Fluke 112). Because of high dependency between temperature and optical output power the LEDs were cooled using air fans during the treatment. Input electrical power was calculated from input voltage and current. Ultraviolet irradiance was monitored from using a spectroradiometer (Stellarnet Black Comet) with a cosine receptor (Stellarnet CR2).

A batch type reactor was constructed for the experiments (Fig. 2). It included a LED module, a quartz glass petri dish (Ø 50 mm) with a small magnetic stirrer and power source. A 5 mL sample was poured into the dish and gently stirred during the treatment. Two LED modules were placed 10 mm above and below the sample. UV dosage of the sample was varied via treatment time. Decrease in UV irradiance was not observed when measured through the petri dish or the sample layer with maximum thickness of 1/3 mm. Thus, absorption did not have to be taken into account and an assumption could be made, that the equal dose was introduced across the sample. Peak wavelengths in the LEDs used were 256, 262, 268, 274 and 278 nm with average peak width of 11 nm (Fig. 3). The irradiance values of the emission spectra in figure are represented in relation to the highest peak 278 nm. The four studied combinations were 256 + 262, 262 + 268, 268 + 274 and 274 + 278 nm.

2.3. Treatment efficiency

Treatment efficiency was determined using pulse-amplitude modulated fluorometer (Water-PAM, Walz). The photosynthetic activity was determined by the response of a dark-adapted sample to 2.5 ms saturation light pulse. Dark-adaptation time was one

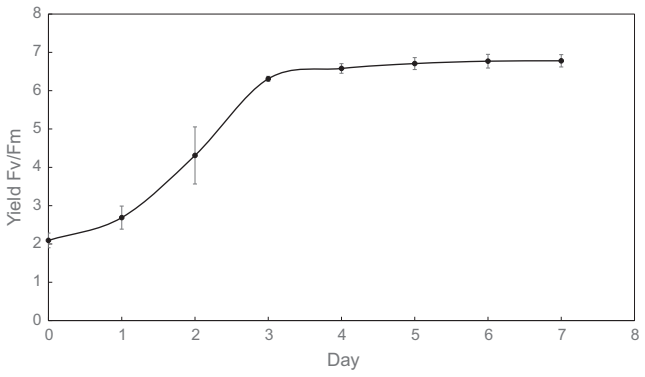

Fig. 1. Representation of 4 first growth phases of studied algacultures, observed using PAM fluorometer yield.

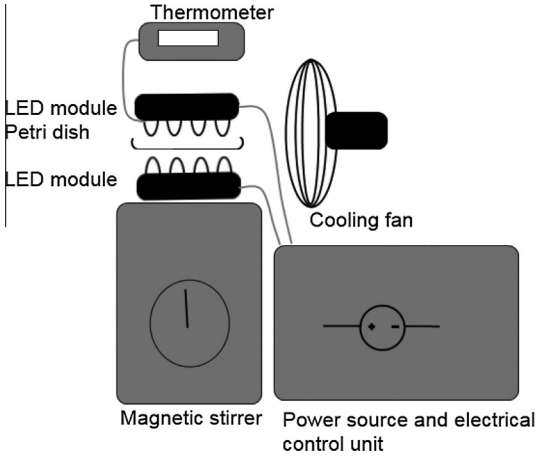

Fig. 2. Schematic representation of the disinfection unit.