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Selective removal of rotifers in microalgae cultivation using hydrodynamic cavitation

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ABSTRACT

Rotifers in algal open ponds consume microalgae rapidly, eventually causing the pond to crash. Hydrodynamic cavitation (HC) has been suggested as a means of controlling rotifers, and its effect on *Nannochlropsis salina* was examined here. Rotifers were removed at a rate of 87% after a single pass of HC when the initial concentration was 1000 individuals/mL, and up to 99% after four passes, regardless of the initial concentration. The removal rate is expected to be higher than 96% with a single pass in an actual pond, as the rotifer concentration does not typically exceed 500 individuals/mL, even under favorable environmental conditions. At the same intensity of HC, the reproductive capability of *N. salina* dropped by 12–15% however, the growth exhibited a constantly increasing overall tendency. In addition, the applied HC process was found to be the most energy efficient approach among the existing physical methods for controlling zooplanktons, requiring 6 MJ of energy for treating 1 m^3 of algal suspension. Rotifers were sufficiently vulnerable and algae were relatively tolerant to the HC, and therefore HC can be adopted as a selective crop-protection method in microalgae cultivation.

1. Introduction

Open ponds are common microalgae cultivation systems, and they provide a practical solution for biofuel production and wastewater treatment. However, given that the ponds are exposed to the atmosphere, invasions of other microorganisms such as bacteria and predators can be particularly detrimental [1]. Omnivorous zooplankton grazers are predators of microalgae, consuming microalgae biomass at exceedingly high rates; e.g., a single rotifer can consume 0.1–0.3 million cells of microalgae per day [2]. When rotifers thrive, algae cannot grow and will form algal flocs [3,4], and the pond eventually can crash. Therefore, effective control of zooplankton grazers including rotifers is imperative for stable pond operation and the protection of biomass.

A number of crop protection methods using various physical, chemical, and biological mechanisms have been suggested. Chemical biocides are well developed and commercially available, but the residual activity on microalgae is a potential concern when taking into account the downstream processes such as lipid extraction and catalytic conversion [5]. Biological methods, which introduce predatory organisms of zooplanktons, have only been studied in natural and aquaculture systems, and are difficult to control given the limited understanding as the ecosystem is more complicated in the pond [1]. Among the physical methods, cavitation by using sonication is known to be effective, but application to a large-scale operation is challenging [6].

Hydrodynamic cavitation (HC) has been suggested as a simple and economical means of eradicating zooplanktons during the treatment of wastewater [7]. HC can be realized simply by changing the cross-sectional area of a pipe carrying a flowing liquid to induce a rapid pressure drop and recovery. During this flow change, micro-sized bubbles are generated and subsequently explode, and extremely high shear stress with strong turbulence and shock waves is brought about, resulting in an instant rise in the local temperature in a range of 500–15,000 K with pressure of 100–5000 atm [8]. This destructive force has been utilized in wastewater treatment processes to destroy unwanted microorganisms or to enhance the downstream anaerobic digestion of activated sludge [8,9]. However, no systematic studies that would facilitate the use of HC for rotifer control in algal ponds have been reported to date.

This paper presents a proof of concept that demonstrates the

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possibility of HC-based rotifer control. The removal capability of the HC process was measured under concentrated rotifer concentrations with cumulative cycles, and its effect on the rotifers and the eggs was observed. The effect of HC on *N. salina* was also evaluated, primarily based on the algal biomass productivity. The utility of HC as a crop protection method was compared with that of other previously reported methods in terms of removal rate and energy consumption.

2. Material and methods

2.1. Preparation of rotifers and microalgae

A type of rotifer (*Brachionus rotundiformis*) was purchased from Aquanet in South Korea. A green microalgae species, *Nannochloropsis salina*, was cultivated with a modified f/2 medium [10] that contained a fivefold higher concentration of sodium nitrate. The medium consisted of sea salts (30 g/L), $NaNO_3$ (375 mg/L), $NaH_2PO_4{\cdot}9H_2O$ (5 mg/L), $FeCl_3{\cdot}6H_2O$ (3.15 mg/L), $Na_2EDTA{\cdot}2H_2O$ (4.36 mg/L), $CuSO_4{\cdot}5H_2O$ (9.8 μg/L), $Na_2MoO_4{\cdot}2H_2O$ (6.3 μg/L), $ZnSO_4{\cdot}7H_2O$ (22 μg/L), $CoCl_2{\cdot}6H_2O$ (10 μg/L), $MnCl_2{\cdot}4H_2O$ (180 μg/L), vitamin B12 (0.5 μg/L), biotin (0.5 μg/L), and thiamine hydrochloride (100 μg/L). The cultivation was maintained in a 10-L flat panel photobioreactor (PBR) for 7 days at 25 °C. The PBR was constructed with PVC for the body and polycarbonate sheets for the panels. Light was supplied, and its intensity was increased in a stepwise manner from 100 to 400 μmol/m^2. CO_2 (2%, 0.5 vvm) was supplied.

2.2. Hydrodynamic cavitation

The HC device used in this study consisted of a multi-stage centrifugal pump (TPH2T6KS; Walrus Pump (Taiwan) Ltd.), stainless pipes (Φ 2 cm, SUS 316), an orifice plate (Φ 2 cm), and a reservoir (20 L, SUS 316) (Fig. 1). The orifice plate had 27 holes, each 0.5 mm in diameter, and its opening ratio (total opening area of the orifice/cross-sectional area of the pipe) was 0.016. The pressures upstream and downstream of the orifice were maintained as 3 and 0.3 bars, respectively. The fluid velocity at the orifice plate was 18.86 m/s. The cavitation number (CN) is defined as:

$$CN = 2(P_u - P_d)/\rho v^2 \quad (1)$$

where P_u is the upstream pressure, P_d is the downstream pressure, ρ is the density of the fluid, and v is the fluid velocity at the orifice [8]. The cavitation number in this experiment was calculated to be 1.52, falling into a range where cavitation inception occurs (1–2.5) [11].

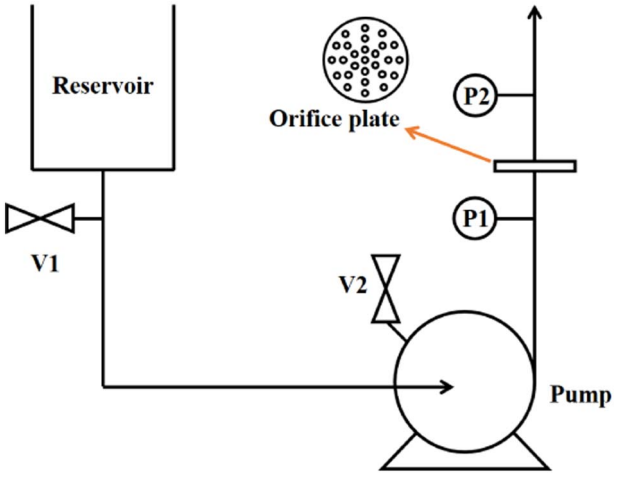

Fig. 1. Schematic diagram of hydrodynamic cavitator. (P: pressure gauge, V: valve).

2.3. Timing of HC treatment

HC with intensity as described in Section 2.2 was applied to rotifers and microalgae. In the case of rotifers, four samples were prepared, with initial concentrations of 1000, 2500, 5000, and 8000 in duplicate. Each sample underwent four passes of HC, and the number of living rotifers between the passes was counted. Microalgae cells, grown in the PBR for 7 days, were subjected to four passes of the HC, and then returned to the PBR. Approximately 100 mL of culture solution was taken (i) before the HC, (ii) after a single pass, and (iii) after the four passes to create three groups (control, group1, and group 2), as described in the following section.

2.4. Viability test of treated microalgae in baffled flasks and PBR

After the HC treatment, the viability of the microalgae was evaluated by cultivating *N. salina*. To examine the comparative effect of HC on *N. salina*, three groups were prepared in quadruplicate: a control group for which HC was not used; group 1, which underwent HC once; and group 2, which underwent HC four times. All three groups were inoculated in a 250-mL baffled culture flask with the modified f/2 medium, and the working volume was 120 mL. The temperature of the incubator was maintained at 25 °C, with CO_2 (2%, 0.5 vvm) and light (120 μmol/m^2·s) supplied, and the bottom plate holding the flasks was rotated at 120 rpm for mixing. The initial concentration was 0.1 for the optical density at a wavelength of 680 nm. After 7 days of cultivation, the final cell density (cell numbers in unit volume), the dry cell weight, and the quantum yield were compared. Another viability test was conducted in duplicate using the PBR, and the conditions used in this test were identical with those noted in Section 2.1, except that the cultivation time was 12 days. During the cultivation, all cells including the medium underwent the HC process four times after 7 days and were then returned to the PBR to continue the cultivation for an additional 5 days.

2.5. Analytical methods

2.5.1. Counting rotifers

A reservoir containing the rotifers and a medium was vigorously mixed and 15 mL of the sample was then taken. Before counting the rotifers, the sample was stored in a refrigerator (− 4 °C) for 1 h to slow the movement of the rotifers. Rotifers that were partially damaged and thus unable to swim were also counted as long as they maintained their original shape. The number of rotifers was counted with a hemocytometer (plankton chamber with grids, MJB & I, Republic of Korea) using a microscope (Eclipse TS100, Nikon), which was also used to obtain images of the rotifers. All data were reported as the mean value of two different reservoirs.

2.5.2. Dry-cell weight and cell density of microalgae

The flask containing *N. salina* and the medium was vigorously mixed and 5 mL of the sample was then taken. The dry cell weight was measured by filtering the samples on a pre-weighed and pre-dried 0.45 μm cellulose nitrate membrane filter (Whatman, USA). The cells were dried in an oven at 65 °C for 24 h to evaporate all of the water content. Microalgal cell density was estimated by counting cells with a disposable hemocytometer (DHC-N01 (Neubauer Improved), INCYTO, Republic of Korea) using a microscope (Eclipse E200, Nikon), which was also used for observing the images of *N. salina*. All data were reported as the mean value of four different culture flasks.

2.5.3. Quantum yield

The baffled flask containing *N. salina* and the medium was vigorously mixed and 5 mL of the sample was then taken. The sample was placed in a dark room for 60 min to ensure that *N. salina* were adapted to a dark state. A fluorometer (Aquapen AP-C100; Photosystems

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