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Foam flotation can remove and eradicate ciliates contaminating algae culture systems

Abbas Umar[a,*], Gary S. Caldwell[b], Jonathan G.M. Lee[a]

[a] School of Chemical Engineering and Advanced Materials, Newcastle University, Merz Court, Claremont Road, Newcastle upon Tyne, NE1 7RU, UK
[b] School of Marine Science and Technology, Newcastle University, Ridley Building, Claremont Road, Newcastle upon Tyne, NE1 7RU, UK

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ABSTRACT

We demonstrate, for the first time, the efficacy of a surfactant-aided foam flotation system to remove and eradicate ciliates contaminating microalgae cultures. Using sodium dodecyl sulphate (SDS) as the surfactant, ciliates removal efficiencies of up to 86.6% were achieved from pure ciliates cultures at an SDS concentration of 40 mg L^{-1}. At this concentration the majority of ciliates were lysed due to increase in SDS concentration in the collected foamate. The removal efficiency decreased to 55.0% in mixed algae-ciliates cultures however, this was compensated by employing a multistage flotation and SDS (50 mg L^{-1}) reuse strategy that achieved removal efficiencies of 96.3%, lysing all collected ciliates, but not affecting microalgae growth. The chemicals cost for the process was US$ 0.0025 per m^3 substantially less than comparator treatments. Building upon its applications in biomass dewatering, pre-processing and sterilising, we add metazoan contamination control to the utilitarian properties of foam flotation for the microalgae biotechnology sector.

1. Introduction

The industrial scale production of microalgae typically occurs in open culture systems such as ponds and raceways and in closed photobioreactor systems. Photobioreactors afford the grower much greater control over the culture environment, whereas open culture systems are, to all intents and purposes, a microcosm of natural waterways; incorporating the risks posed to microalgae from pathogens and grazers. In natural systems microalgae blooms are kept in check by an assortment of biotic actors, including; viruses [1], bacteria [2], parasites [3], protists (including ciliates) [4,5], zooplankton [6], and fungi [7]. However, such biological control can be catastrophic for algae culture systems, particularly when attempting to culture at industrial scale.

Reflecting the equivalence to natural systems, the type of contaminants found in ponds and photobioreactors (the ubiquitous bacteria and viruses aside) include; parasites [8], protists [9], fungi [10], amoebae [11], and rotifers [12]. There is therefore an imperative to develop cost-effective, robust and scalable culture management strategies that either prevent contamination, severely limit the population growth of the contaminant, and/or can safely remove the contaminating agent without harming the microalgae or necessitating major disruption to culture operations. This desire for contaminant control has vexed mass algae growers for over half a century [13–15] and the microalgae industry continues to wrestle with contaminant management [16–18], spawning growing research fields in monitoring and modelling of contamination scenarios [19–24].

Current approaches to culture management include growing species or strains that tolerate abiotic factors (e.g. temperature and salinity) out with the tolerance range of their predators or competitors; *Dunaliella salina* culture being a prime example [25]. Rather more interventionist methods include dosing with chemicals such as pesticides [26–28] and disinfectants [29,30]; however, there remains the risk of causing inadvertent harm to the algae [31], or worse, to the culture personnel. Alternative ecological-based control strategies have been investigated [32–35].

Foam flotation, a technique to separate solution components (including microalgae [36]) by exploiting the variation in their surface charges, is a hitherto unexplored technology for contaminant removal. The closest approximation is that of Kamaroddin et al. [37] who used a microbubble-driven airlift loop bioreactor, switching between CO_2 and ozone as carrier gases, to demonstrate that it was possible to both disinfect and harvest *D. salina* cultures. Building upon work in surfactant-aided foam flotation which demonstrated the efficacy and economic merits of the approach for dewatering and pre-processing microalgae biomass [38–40], the use of foam flotation for the removal of contaminating ciliate from cultures of *Chlorella vulgaris* was investigated.

* Corresponding author.
E-mail address: a.a.umar1@ncl.ac.uk (A. Umar).

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2. Materials and methods

2.1. Microalgae and ciliates culture

Chlorella vulgaris (CCAP 211/63) was cultured in BG-11 medium in 10 L polycarbonate carboys (Nalgene) at 18 ± 2 °C and a 16 L: 8D photoperiod with a mean luminance of 2500 Lux. The cultures were continuously aerated using an aquarium pump (Blagdon KOI AIR 50). The microalgae were grown for three weeks, reaching a cell density of $3.28 \times 10^7 \pm 10^6$ cells mL^{-1}.

The ciliate *Tetrahymena pyriformis* (CCAP 1630/1W) was purchased from the Culture Collection of Algae and Protozoa, UK. *T. pyriformis*, despite not being a microalgae grazer, was chosen for this lab test as it is a well-researched organism that may be considered as a model to begin evaluating and optimizing our foam column separation technique, which can subsequently be extended to other protozoa species that pose a threat to microalgae cultivation. One of the benefits of using *T. pyriformis* is its rapid growth rate, with a population doubling time of 5–7 h under optimal conditions [41]. The culture was grown axenically at 18 ± 2 °C, pH 6.5–7.5 in autoclaved proteose peptone yeast extract medium (PPY) containing 2% proteose peptone and 0.25% yeast extract (Sigma Aldrich, UK). The medium was stored at 4 °C and subsequently adjusted to 15 °C prior to use. A dense *T. pyriformis* culture was gently agitated to evenly mix the ciliates and 0.5 mL was transferred to 10 mL test tubes containing 5 mL of PPY medium using a sterile Pasture pipette. After seven days the density of each new culture was checked using an LEICA DM500 inverted microscope at × 200 magnification. The test tube junction cap was subsequently wrapped with white plastic nylon to reduce evaporation.

2.2. Foam flotation column

The foam flotation column was a modified version of that described by Coward et al. [38] (Fig. 1); briefly, a sparger made from 6.0 mm thickness fine grade polyethylene sheet was used to provide bubbles with an average diameter of 1.13 ± 0.14 mm. The column section was made from a series of modular sections of 250 mm in length, 47.5 mm internal and 51.5 mm external diameter. The column height was 510 mm. A collection cup was attached to the top of the column to receive the foam. The total column volume was approximately 1.0 L. The foam harvester was designed as a foam separation column for the physical separation of the ciliates.

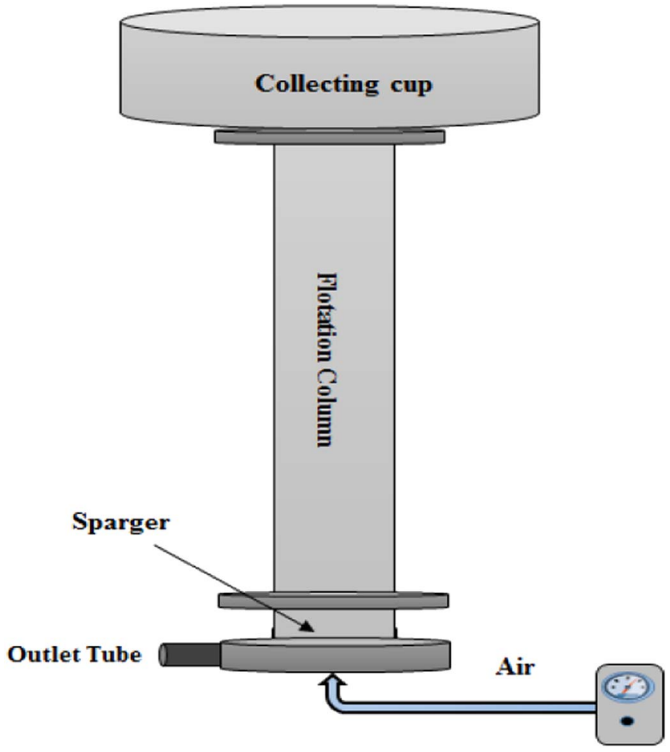

Fig. 1. The foam flotation column dimensions: foam collection cup, 300 mm diameter, 100 mm tall; flotation column, 510 mm tall, 51.5 mm outer diameter, 47.5 mm inner diameter; polyethylene sparger, 6.0 mm thickness.

2.3. Ciliates multistage harvesting using the foam column

Three sodium dodecyl sulphate (SDS) concentrations (20, 30 and 40 mg L^{-1}; Sigma Aldrich, UK) were made in reverse osmosis water and tested in a foam column operating in batch mode. Two experiments were conducted at airflow rates of 1.0 and 2.0 L min^{-1}. Air was fed to the sparger to produce bubbles which led to the formation of a surfactant stabilised foam. The foam flowed up the column and was collected in a collection cup. Before each trial, 50 mL of *T. pyriformis* culture was added to 950 mL of SDS solution. To ensure sufficient space within the column for foam to form, each run consumed 250 mL of the SDS-ciliates solution; allowing for four replicate runs per condition. The foam collected from each experiment was allowed to collapse, was collated and the volume determined. The *T. pyriformis* cell density in the foam and the remaining liquid phase was measured using an improved Neubauer haemocytometer and an inverted light microscope. A drop of Lugol solution was used to immobilise the ciliates for counting. Each column run lasted for a maximum duration of 30 min or until it was no longer possible to collect any more foam, whichever came first. The collated foam (ca. 10 mL) with an assumed high concentration of recovered SDS (including ciliates) was returned to the culture liquors and a further run was conducted as described above. In total, three runs were conducted per SDS concentration as part of a multistage ciliates harvest (the fourth run did not produce any further foam). The aforementioned experiments were repeated but with the addition of *C. vulgaris* ($3.28 \times 10^7 \pm 10^6$ cells mL^{-1}). To control for any effect of air pressure on the ciliates, 100 mL of *T. pyriformis* culture was transferred into the foam column and subjected to 2 L min^{-1} air flow without any SDS for 30 min. The numbers of ciliates in the foamate and the Removal Efficiency (RE) were determined using Eqs. (1) and (2) respectively.

$$\text{Ciliates in foamate} = A - B \text{ cells mL}^{-1} \quad (1)$$

where: A is the initial ciliates count prior to each run, and B is the final ciliates count after each run.

$$\text{RE} = \frac{\text{Ciliates in foamate}}{\text{Initial ciliates count}} \times 100 \quad (2)$$

2.4. Determining the effect of SDS on the ciliates

Initial observations during harvesting suggested that the highest SDS concentration caused the ciliates to lyse; therefore an experiment was conducted to determine the lowest SDS concentration beyond which the ciliates could not survive. Four SDS concentrations (40, 44, 48 and 52 mg L^{-1}) were prepared and added to 1 L of algae/ciliates culture. The ciliates were monitored on an hourly basis using an inverted microscope and a haemocytometer.

2.5. Multistage foam column ciliates eradication using high SDS concentration

Twenty millilitres of *T. pyriformis* (ca. 10,000 ± 10^3 cells mL^{-1}) was added to 980 mL of *C. vulgaris* and mixed with 50 mg SDS. The mixture was allowed to stand for 2 h. A control sample was also prepared without SDS. The concentrations of both *C. vulgaris* and *T. pyriformis* prior to, and after the experiment were determined. The foam column described previously was used to recover the SDS (ca. 10 mL)