Algal Research 32 (2018) 321–328

Contents lists available at ScienceDirect

Algal Research

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

Microalgal CO_2 capture at extreme pH values

Jonna Piiparinen[a], Dorothee Barth[b], Niels T. Eriksen[c], Sebastian Teir[b], Kristian Spilling[a], Marilyn G. Wiebe[b,*]

[a] Finnish Environment Institute (SYKE), Marine Research Centre, P.O. Box 140, FI-00251, FI-00560 Helsinki, Finland
[b] VTT Technical Research Centre of Finland Ltd., P.O. Box 1000, FI-02044 VTT Espoo, Finland
[c] Department of Chemistry and Bioscience, Aalborg University, Fredrik Bajers Vej 7H, DK-9220 Aalborg, Denmark

ARTICLE INFO

Keywords:
Microalgae
pH
CO_2 uptake
Bicarbonate
Specific growth rate

ABSTRACT

Although algae are often grown at pH values between 6 and 8, shifting to more alkali or acidic conditions may benefit CO_2 delivery to algal cultures. To assess the impact of culture pH on growth rate and uptake of CO_2, we grew three relatively fast growing acidophilic (*Coccomyxa* sp., *Euglena mutabilis* and *Euglena gracilis*) and three alkaliphilic (*Thalassiosira pseudonana*, *Phaeodactylum tricornutum*, *Chlamydomonas* sp.) algal species at pH values near neutral and near the extreme of their growth range. All six species showed similar growth and CO_2 uptake ability at extreme as at neutral pH values. Cultures of the alkaliphilic species captured a higher proportion of CO_2 from the gas stream than the acidophilic species; removing 50 to 65% of CO_2 from air compared to only 38% removed by acidophilic species (or 10–24% from CO_2 enriched air). Alkaliphilic species did not become carbon limited when fed CO_2 at the concentration provided by air (0.04% CO_2), but produced less biomass and captured less total CO_2 (0.06 to 0.08 g CO_2 per day) than the acidophilic species (0.6–0.8 g CO_2 day^{-1}) which required CO_2 enriched air to avoid carbon limitation. Bicarbonate feeding reduced the loss of CO_2 to the environment, compared to feeding gaseous CO_2, but with a potential cost in reduced specific growth rate or biomass production.

1. Introduction

The ability of algae to use waste CO_2 and thus reduce direct CO_2 emissions provides a strong motivation for development of large scale algal cultivation. CO_2 is taken into a cell, either as CO_2 or as bicarbonate, and the carbon is converted into biomass or other products through photosynthesis, releasing oxygen to the atmosphere. The potential for capture of CO_2 by cultivating algae is generally agreed to be higher than that from cultivating land plants, at least for those algal species which have high growth rates [1,2]. Many algal species grow under conditions such as high salinity, which are not suitable for plants, adding to the interest in algal cultivation. Algal capture of carbon results in temporary storage, the carbon being converted into products such as food, nutritional supplements, fuel, animal feed or fertiliser.

Air can be used to provide CO_2 for algal growth, but CO_2 becomes limiting unless the mass transfer coefficient k_La value of the reactor is high. A more concentrated source of CO_2 can improve growth also at low k_La values. Algal cultivation should thus utilise waste CO_2 from neighbouring industries. This raises the question of how CO_2 can best be fed to the algal cultures. CO_2 may be injected as a gas to the culture, contributing to the mixing, it may be dissolved into fresh cultivation media using a separate absorption column, or it can be added in alkaline solution as bicarbonate [3]. The delivery system both affects and is affected by the pH of the cultivation. Direct injection of CO_2 lowers the pH of aqueous systems [4,5]. In contrast, when CO_2 from flue gas is captured as bicarbonate, high pH values are needed to ensure that the bicarbonate remains in solution and is not released to the atmosphere. Alkaliphilic algae that sustain high specific growth rates over a pH range of 7 to 10 can be used when supplying CO_2 as bicarbonate using an alkali scrubber to initially capture the CO_2 from the flue gas [6].

In this paper we evaluate the growth and CO_2 uptake of three relatively fast growing acidophilic (*Euglena gracilis*, *Euglena mutabilis* and *Coccomyxa* sp.) and three alkaliphilic (*Thalassiosira pseudonana*, *Phaeodactylum tricornutum*, and *Chlamydomonas* sp.) algae at an extreme pH, relative to neutral pH, and consider the implications for methods of CO_2 provision from industrial sources. Algae of these genera have been reported to have specific growth rates of 0.72 (*E. gracilis*; [7]), 0.58 (*E. mutabilis*, [7]) and 0.70 (*Coccomyxa onubensis*, [8]) day^{-1} at pH 3 and 1.8 (*T. pseudonana*; [9]), 0.3 (*P. tricornutum*, [10]) and 0.41 (*Chlamydomonas* sp., [11]) day^{-1} at pH 9. The potential for bicarbonate feeding to reduce loss of CO_2 to the atmosphere is also demonstrated.

* Corresponding author: VTT Technical Research Centre of Finland Ltd., P.O. Box 1000, FI-02044 VTT Espoo, Finland.
E-mail address: marilyn.wiebe@vtt.fi (M.G. Wiebe).

https://doi.org/10.1016/j.algal.2018.04.021
Received 28 November 2017; Received in revised form 28 March 2018; Accepted 24 April 2018

2. Material and methods

2.1. Strains and media

Euglena mutabilis (CPCC452) and *Coccomyxa* sp. (CPCC508) were purchased from the Canadian Phycological Culture Centre (CPCC). *Thalassiosira pseudonana* (CCMP1335) and *Chlamydomonas* sp. (CCMP2294) were purchased from the National Center for Marine Algae and Microbiota (NCMA) – Bigelow Laboratory for Ocean Sciences. *Euglena gracilis* (CCAP1224/5Z; equivalent to UTEX753) and *Phaeodactylum tricornutum* (CCAP1055/1) were obtained from The Culture Collection of Algae and Protozoa.

All algae except *Chlamydomonas* sp. CCMP2294 were maintained in chemically defined medium at room temperature (~22 °C) or 16 °C in stationary or agitated (90 rpm) flasks near a light source (8 W, providing 100 to 150 μmol photons $m^{-2} s^{-1}$ at the flask surfaces) with 14–16 h light and 8–10 h dark. *Chlamydomonas* sp. CCMP2294 was maintained in stationary flasks at 4 °C with variable light (approximately 14 h dark, 10 h light).

Acidophilic algae were grown in the Modified Acid Medium (MAM) recommended by CPCC [12]. Alkaliphilic algae were grown in L1 medium (diatoms) [13], L1 lacking silicate (*Chlamydomonas* sp. CCMP2294), or modified f/2 medium (T2) designed to provide N, P and Si in a ratio of 16:1:8 [14]. *P. tricornutum* growing in L1 in the photobioreactor was provided extra N, P, Si and vitamins in cultures in which these became limiting. Tropic Marin Sea Salt™ was used to provide 28–30 g L^{-1} salt for marine species (*T. pseudonana*, *P. tricornutum*, *Chlamydomonas* sp. CCMP2294).

In photobioreactors, phosphorus was also provided as H_3PO_4 (0.5 M) through pH regulated titration. For cultures which received CO_2 as bicarbonate, this was fed in 0.5 M solutions as either sodium bicarbonate or ammonium bicarbonate.

2.2. Culture conditions

Inocula for preliminary cultivations were grown in 50–500 mL volumes at 20 °C and were provided 100 μmol photons $m^{-2} s^{-1}$ in a 16 h light and 8 h dark cycle. Alternatively, inocula were grown in 15 to 50 mL medium in 50, 100 and 250 mL Erlenmeyer flasks on a shaking platform (Infors AG Switzerland) at 90 rpm, at room temperature, with one tubular fluorescent light (8 W) placed beside the flasks with a 14 h light, 10 h dark light cycle (light, 400 to 700 nm, was supplied at 100 to 150 μmol photons $m^{-2} s^{-1}$ on culture surfaces). *Chlamydomonas* sp. CCMP2294 was grown in static conditions at 4 °C.

For preliminary measurements of specific growth rate and pH tolerance, algae were grown in 2 L Nalgene flasks placed in a water bath at 23 °C with ca. 100 μmol photons $m^{-2} s^{-1}$ in a 16 h light/8 h dark cycle (*T. pseudonana*, *P. tricornutum*, *E. gracilis* and *Coccomyxa* sp. CPCC508, in triplicate) or in continual light (*E. gracilis* and *Coccomyxa* sp. CPCC508, single cultivations). The cultures were semi-continuously bubbled with 0.2 μm filtered air at a rate which provided mixing without affecting culture pH (1 min air every 1.2 h). The pH of the cultures was monitored 1–2 times a day with a Metrohm 780 pH-meter and adjusted manually with HCl (0.1–2 M) or NaOH (0.1–0.5 M). The cultivation time varied from 6 to 14 days, depending on the species and pH. The flasks were inoculated to provide an initial density of ca. 1000 cell mL^{-1}, with sequential studies of growth at pH values of 3, 4.5 (pH of MAM medium), 6 and 7.3 (acidophilic species) or pH 8.5 (pH of T2 medium), 9, 9.5 and 10 (alkaliphilic species). Cell growth was monitored until the end of the exponential growth phase and flasks were emptied and re-filled, leaving an inoculum of ca. 1000 cells mL^{-1}, when the cultures were no longer growing exponentially.

For simultaneous determination of growth and CO_2 utilisation, cells were cultivated in continuous light in Sartorius Biostat B 2.5 L glass vessel bioreactors (Sartorius AG, Germany). Biostat B cultures were maintained at pH values of 3, 6, 7 to 7.5 or 9, as described in the results, by the addition of 0.5 M H_3PO_4 or 0.5–1.0 M NaOH, with 200 rpm agitation, at 23 °C (4 °C for *Chlamydomonas* sp. CCMP2294). Biostat B reactors were placed between two LED Light Source panels (one 20 × 20 cm and one 20 × 30 cm panel per reactor; SL 3500 from Photon Systems Instruments; Drasov, Czech Republic). Light was provided from a mixture of blue, green and red LEDs, giving between 150 and 600 μmol $m^{-2} s^{-1}$ at the inner surface of the reactor facing the panels and 50 to 175 μmol $m^{-2} s^{-1}$ at the surfaces furthest from the light panels. Light was increased as the algae grew, to reduce the extent to which light would limit growth while avoiding light inhibition during the initial stages of growth. Light was measured with a quantum sensor connected to a radiometer (Li-Cor. Inc. USA). Bioreactor cultures were grown as repeated batch cultures to reduce the lag phase during which the algae adapted to the reactor environment. To initiate a new culture, approximately 95% of the culture volume was removed and replaced with fresh culture medium.

Biostat B reactors were used with a 2 L working volume and aerated with 0.3 L air (or total gas) per minute (i.e. 0.15 volume of air per volume culture per minute; vvm). For cultures supplemented with CO_2, CO_2 was provided in a mixture with N_2 (Oy AGA Ab, Finland) and mixed with air to provide between 1.8 and 2.4% CO_2 in the aeration gas. Gas composition (CO_2, O_2, and N_2) in the outlet gas was analysed continuously with a Prima Pro Process mass spectrometer (Thermo Scientific, UK) calibrated with 3% CO_2 in Ar, 5% CO_2 with 0.99% Ar and 15% O_2 in N_2, 20% O_2 plus 20% Ar in N_2, and 0.04% ethanol in N_2.

For bicarbonate feeding, air was passed through the head space of a Biostat B reactor at 0.05 L per minute (0.025 vvm) in order to measure CO_2 released from the medium into the gas phase. No air was sparged through the liquid. Bicarbonate was fed as either 0.5 M sodium or ammonium bicarbonate at rates between 1.0 and 1.5 mL h^{-1}. The feed rate was adjusted to obtain the highest possible carbon input, while minimising the loss of CO_2 from the system. In practice this meant that the feed rate was reduced when the CO_2 concentration in the output gas was above that in air, and was increased when the concentration was equal or lower to that in air. Cultures were initially agitated at 200 rpm, but agitation was increased in 50 rpm steps to 350 (*P. tricornutum*) or 400 (*E. gracilis*) rpm to reduce the concentration of oxygen dissolved in the medium.

2.3. Analyses

2.3.1. Cell biomass

Cell biomass was measured as cell number (fixed with acid Lugol's solution), optical density at 780 nm (OD_{780}) to avoid interference from photosynthetic pigments, and/or dry biomass (DW). Cells were counted using a Leica DMI3000 B inverted microscope with 200–630× magnification. Cell numbers were used to determine specific growth rates when assessing the pH range for growth. OD_{780} was measured in duplicate. For DW determination, 2 to 50 mL culture was centrifuged (3600 rpm, 6 min for large volumes, 13,000 rpm for 3 min for 2 mL volumes), and the pellet was washed twice with Milli-Q® water. During the washing steps, the cell biomass from large sample volumes was transferred to a 2 mL pre-dried (105 °C) and pre-weighed microcentrifuge tube, in which it was taken to dryness at 105 °C. The weight of unwashed, dried biomass that was used to determine the elemental composition of the algae demonstrated that loss of biomass to lysis during washing steps was minimal (data not shown). Relationships between OD_{780} and DW for the 6 algae are given in Table 1. Biomass in the CO_2 uptake experiments was primarily measured as OD_{780}, from which the biomass concentration (DW) was calculated. Since the OD_{780}-DW relationship is not necessarily constant throughout the growth cycle, specific CO_2 uptake is an estimate. For *P. tricornutum*, *E. gracilis* and *Chlamydomonas* sp. CCMP2294 the relationship between cell number and OD_{780} was also assessed and showed good correlation (Table 1).