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Control of the pH for marine microalgae polycultures: a key point for CO₂ fixation improvement in intensive cultures

Amandine Galès¹², Sébastien Triplet³, Thibault Geoffroy³, Cécile Roques¹, Claire Carré¹, Emilie Le Floc’h¹, Mélissa Lanfranchi¹³, Monique Simier¹, Emmanuelle Roque d’Orbcastel¹, Cyrille Przybyla¹, Eric Fouilland¹*

¹MARBEC, Univ Montpellier, IRD, CNRS, IFREMER, Montpellier, Sète & Palavas, France
²LBE, Univ Montpellier, INRA, Narbonne, France
³IFREMER L-SEA, Palavas, France
*Corresponding author: eric.fouilland@cnrs.fr

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Abstract

Recently, CO₂ recycling for the production of valuable microalgae has acquired substantial interest. Most studies investigating CO₂ conversion efficiency in algal cultures were based on single species, although a stabilising effect of algal diversity on biomass production was recently highlighted. However, addition of CO₂ into polyalgal cultures requires a careful control of pH; performance of CO₂ conversion, growth and carbon biomass production are affected by pH differently, depending on the species of microalgae. This study investigates the efficiency of CO₂ conversion by natural marine algal assemblage cultivated in open, land-based raceways (4.5 m³, 10 m²), working as high rate algal ponds (HRAP). Ponds were enriched with nitrogen and phosphate, pure CO₂ was added and algal cultures were
grown under three different fixed pH levels: pH 6, 7 and 8. The highest conversion of photosynthetically fixed CO$_2$ into carbon biomass (40%) was reached at pH 7, an intermediate level, due to the partial CO$_2$ asphyxiation of algal predators (copepods, ciliates), while being under the suboptimal conditions for the development of marine amoebae. Under this pH, the theoretical maximal biological conversion of available CO$_2$ into carbon biomass was estimated to be 60% in naturally inoculated open ponds.

1. Introduction

Algae has become a focal point in the circular economy to valorise industrial waste in order to continually use resources and reduce industrial CO$_2$ emissions to the atmosphere (Lai et al. 2019; Stiles et al. 2018). Through photosynthesis, microalgae can convert CO$_2$ from industrial flue gas into biomass that can potentially be used for the production of biofuel, biochemical products or green chemistry (Bhola et al. 2014; Brune et al. 2009; Wang et al. 2008). Industrial flue gas contains potentially toxic contaminants which can lead to reductions in the production of biomass (Hess et al. 2017). Additionally, injection of CO$_2$ acidifies algal culture medium by modifying the balance of carbonates in the water (Galloway and Krauss 1961). This acidification could counterbalance CO$_2$ fixation by microalgae which alkalinises their growth environment (Galloway and Krauss 1961). A controlled injection of CO$_2$ would maintain an optimal pH level that could be adjusted to optimise for metabolic activity of algal species (Galloway and Krauss 1961). For example, the marine microalgae *Nannochloris* sp. achieves maximal growth rate at pH 6, maximum carbon content at pH 7 and maximal carbon conversion efficiency at pH 8 (Vasseur et al. 2012). Other example, the optimal growth of the cyanobacteria *Spirulina platensis* and the green algae *Chlorococcum littorale* are 9 and 4, respectively (Zeng et al. 2011). In microalgal predators, lower pH (associated with CO$_2$ injection) negatively affects the densities and grazing activity of rotifers (Montemezzani et al.)
If we ignore the potential effects of associated contaminants, using CO$_2$ from industrial flue gas can combine pH control during high photosynthetic CO$_2$ fixation and reduction of algal grazing pressure.

Large-scale industrial cultivation of marine microalgae appears to be one of the most promising approaches of sustainable sources of food (lipids and proteins) and energy (Greene et al. 2016). Narwani et al. (2016) demonstrated stabilising effects of biodiversity on biocrude production from polycultures of several assembled algal species using raceway ponds under variable environmental conditions. However, higher performance can be reached using natural algal polyculture than assembled polyculture (Thomas et al. 2019).

We suggest that an optimal pH can be reached for maximising carbon conversion efficiency of a natural marine polyculture of microalgae while reducing grazers development.

Natural marine polycultures were cultivated in High Rate Algal Ponds (HRAP) in a Mediterranean climate with different levels of pure CO$_2$. We measured algal diversity, photosynthetic CO$_2$ uptake, carbon biomass accumulation, and associated development of algal predators under three different pH values. We developed performance curves of biological CO$_2$ conversion efficiency a function of pH. Results from this study provide a comprehensive assessment of the biological CO$_2$ conversion efficiency from marine microalgae cultivated in open ponds. The results from this experiment can inform engineering projects for industrial CO$_2$ sequestration or mass production of marine microalgae.

2. Material & Methods

2.1. Experimental design

An intensive culture of natural microalgae from Mediterranean seawater was performed outdoors in high rate algal ponds (HRAP) as described in Metaxa et al. (2006). HRAPs are oval-shaped ponds (6 m long, 2 m wide and 0.6 m deep) with a working volume
of 4.46 m$^3$ and surface area of 10 m$^2$. Water mixing in the HRAPs was maintained at 0.2 m s$^{-1}$ using a vacuum airlift column developed and patented by COLDEP® (Barrut et al. 2013). Briefly, the column was connected to the HRAP and was composed of a central tube, the top of which was hermetically closed and connected to a vacuum pump. Water was raised to top of the central tube with a vacuum and was allowed to flow over the central tube so that it could be returned to the HRAP. We maintained pH using an automatic JBL pH-controller system (JBL ProFlora m1003) connected to a pure CO$_2$ bottle using a solenoid valve. We diffused CO$_2$ through a ceramic gas diffuser in the HRAP. The JBL ProFlora system used a combined Ag/AgCl KCl-electrolyte pH electrode at operational IUPAC scale calibrated monthly using commercial NIST buffers with precision of 0.05 pH units with an additional systematic error of about 0.15 pH units relative to seawater scale (Riebesell et al. 2011).

Experiments were conducted at the Ifremer Station of Palavas-les-Flots, Hérault, France during spring and early summer 2016 to coincide with the climatic period of favourable algal growth. The first run of experiment (run 1) occurred from 17 May to 22 June 2016 (36 days); run 2 occurred from 27 June to 26 July 2016 (29 days). For each experimental run, three HRAPs were monitored simultaneously on batch feeding mode and maintained at pH of 6, 7 and 8, respectively. We assessed cumulated photosynthetic CO$_2$ fixation, production of carbon biomass, microalgal diversity and predator identification.

For all experiments, seawater was pumped from the Mediterranean Sea, inoculated using non-specific microalgae inoculant and filtered through a 100 µm sand filter before entering HRAPs. All HRAPs were supplemented with phosphorus to have final concentrations of 5 mg L$^{-1}$ of phosphate and nitrogen to achieve 80 mg L$^{-1}$ of nitrate and ammonium.

2.2 Physical and chemical variables
Global, direct and diffuse solar radiation were recorded every hour at the Météo-France station at Fréjorgues airport, 7 km from the experimental site.

2.2.2 Water pH, salinity, light and temperature measurements

Water temperature (accuracy of 0.5°C) in HRAPs were recorded every 10 minutes and salinity (accuracy of 0.1 PSU) in HRAPs was recorded once a day using a PONSEL ODEON X meter. In addition to JBL ProFlora system, water pH was measured every 10 minutes in one point of the well-mixed HRAPs (water circulation of 0.2 m.s⁻¹) using a combined Ag/AgCl KCl electrolyte ODEON electrode with a resolution of 0.01 pH units and an accuracy of 0.1 pH units. The underwater light intensity in HRAPs was recorded every 15 min at three depths (sub-surface, 20 and 40 cm) using waterproof light data loggers (UA-002-64 HOBO, Onset).

2.3 Biological variables

2.3.1 Microalgae biomass and diversity

The algal biomass was assessed daily when possible for Chlorophyll a (Chla) and weekly for particulate organic carbon (POC) measurements. For Chla measurements, between 3 and 800 ml samples (depending on sample loading) were filtered onto 13 mm GF/F fibreglass fibre filters to determine the total Chla concentrations. For size-fractionated Chla measurements, between 50 and 800 ml samples were filtered using 20 µm, 5 µm and 2 µm membrane filters and GF/F fibreglass (0.7 µm nominal pore size). Filters were stored frozen at -20°C until extraction with 5 ml of 100% ethanol. Extractions were performed after ultrasonication in an ice bath followed by overnight extraction at 4°C. Samples were then filtered and extract absorbance was measured at 632, 649 and 665 nm (with absorbance at 750 nm used for a baseline correction) using a Shimadzu UV1800 zeroed with 100% ethanol.
Chlorophyll concentration was calculated according to Ritchie (2008). For the POC measurements, between 5 and 100 mL samples were filtered onto precombusted Whatman GF/F filters and stored at -80°C for analysis. The filters were then dried at 60°C for 24 h, pelleted and analysed using an ANCA mass spectrometer (Europa Scientific). Because of the small volume of samples (<100 ml), we assumed that microalgae were mainly collected and retained onto the filters (nominal pore size of 0.7 µm) and the POC measurement was therefore representative of microalgae carbon biomass. This is supported by positive linear correlation between Chl \(a\) and POC measurements \((r^2 = 0.85, p < 0.001, n=33)\) leading to a Chl:a:POC ratio of 55, similar to the value measured for marine green algae and Prymnesiophyceae in the Mediterranean Sea (Latasa 2005).

Microalgal community structure was determined in all HRAPs at the beginning (Day 0) and at the end of each experimental run (Day 36 and Day 29) using 18S rRNA gene analysis. For each experimental run, 10 mL samples were filtered onto 0.2 µm membranes (PALL Supor® 200 PES) and stored at -20°C for subsequent DNA extractions. The DNA was extracted using DNeasy PowerWater Kit (Qiagen) according to the manufacturer's instructions. The V4 region of the 18S rRNA gene was amplified over 30 amplification cycles at an annealing temperature of 65°C, with forward and reverse primers (5'-

\[\text{CTTTCCCTAAGACGCTTCTTCCGATCTTGCGTAATTCCAGCTCCAA-3'} \text{ and 5'}-\]

\[\text{GGAGTCAGACGGTGTGCTTCCGATCTTTGCCAAATGCTTTCGC}-3', \text{ respectively}\) with their associated linkers. The resulting products were purified and loaded onto an Illumina MiSeq cartridge for sequencing, paired with 300 bp reads following manufacturer's instructions (v3 chemistry). Sequencing and library preparation steps were carried out at the Genotoul Lifescience Network Genome and Transcriptome Core Facility in Toulouse, France (get.genotoul.fr). A modified version of the standard operation procedure for MiSeq data (Kozich 2013) in Mothur version 1.35.0 (Schloss 2009) was used for alignment and
taxonomic outline. Mothur was also used to identify representative sequences of operational
taxonomic units (OTUs).

2.3.2 Algal predator diversity
To characterise the presence and abundance of protozoan and metazoan organisms, 2 L water
samples were filtered sequentially through a 100 µm and 35 µm filters to separate 35 to 100
µm and > 100 µm size fractions, which were then stored in neutralised formalin (4% final
concentration). Protozoan and metazoan taxa from a subsample of the 35 to 100 µm fraction
were identified, sized and enumerated using an Olympus AX70 dissecting microscope with a
Nageotte counting chamber. Protozoan and metazoan taxa from a subsample of the > 100 µm
fraction were identified and enumerated using an Olympus Stereomicroscope SZX7 with a
Bogorov counting chamber.

2.3.3. HRAP photosynthetic CO₂ uptake
The photosynthetic CO₂ uptake by microalgae in HRAPs was computed from the relationship
between biomass-specific gross photosynthetic oxygen production and the light intensity
(photosynthesis-irradiance P-I model). The weekly photosynthetic oxygen production was
measured at the laboratory as detailed below. Algal biomass (Chla) was measured every day
when possible in the HRAPs. Average light intensity received by microalgae was measured
eyery 15 min at mid-depth (0.2 m) in each HRAP.
Samples were collected weekly from HRAPs, distributed to air-tight 30 mL
polycarbonate flasks and placed in a custom-made, controlled-temperature photosynthetron
with a light density gradient up to 400 µmol photons m⁻²s⁻¹ using an array of 4 W white
LEDs. Photosynthetically active radiation (PAR) was measured using a US-SQS/L spherical
quantum microprobe (Walz, Effeltrich, Germany). The temperature was kept constant within
the HRAPs by heating or cooling. Dissolved oxygen (to determine net photosynthesis) was measured using a FireSting optode system (Pyro-Science, Aachen, Germany) every 20 min for 6 h of incubation in all illuminated flasks. Net photosynthetic rate was calculated at all light intensities. Samples were also incubated in the dark at the same temperature to determine respiration rate (oxygen consumption). Gross photosynthetic rates for all light intensities were calculated as the sum of net photosynthetic rates and respiration rates. Gross photosynthetic rates were expressed in carbon units using a photosynthetic quotient of 1 (Falkowski and Raven 1997) and in algal biomass units using Chlorophyll \( \alpha \) measurements. These were plotted against irradiance using the P-I model described by Eilers and Peters (1988) using the phytotools R package (https://cran.r-project.org/package=phytotools). The instantaneous gross photosynthetic CO\(_2\) uptake rates were derived from the P-I model obtained every week for each HRAP, the Chl\( \alpha \) biomass measurement measured every day and the light measurements taken every 15 min at mid-depth during P-I model assessment. For the calculation of instantaneous photosynthetic rates, we assumed there was no significant change of P-I parameter values during a week and the Chl\( \alpha \) biomass during a day.

The total gross photosynthetic CO\(_2\) uptake for HRAPs was calculated as the sum of all instantaneous gross CO\(_2\) uptake rates obtained during the whole experiment in each HRAP.

2.3.3. Prediction of minimal CO\(_2\) losses

We attempted to predict the minimal carbon losses through biological activity (respiration, exudation, predation) using a generalised additive model (GAM) in R (R Core Team, 2019) with the mgcv package (Wood, 2017). Carbon lost through biological activity was calculated as the fraction of gross photosynthetically fixed CO\(_2\) not accumulated into the C biomass over time series from HRAPs at pH 7.
3. Results & Discussion

3.1. Influence of pH on algal diversity and predation

In the pH-6 HRAPs, pH averaged 6.2±0.2 (Figure S1). Lower pH variability was observed in the pH-7 HRAPs (Figure S1), where pH averaged 6.7±0.1 and 7.0±0.1 during run 1 and run 2, respectively. In the pH-8 HRAPs, pH averaged 8.2±0.1 and 7.9±0.1 during run 1 and run 2, respectively. When pooling pH measured during both experimental runs, pH averaged 6.2±0.2, 6.9±0.2, and 8.1±0.2 in HRAPs with pH targeted at 6, 7 and 8 respectively.

The addition of CO$_2$ acidifies seawaters and impacts the growth of marine planktonic organisms found in naturally buffered seawater at pH 8.07 in the Mediterranean Sea (Hassoun et al. 2015). Larger microalgae (> 5 µm, Fig. 1), such as *Chlamydomonas* sp., favoured the growth at pH 6, while smaller microalgae (< 2 µm, Fig. 1), such as *Nannochloris* sp. and *Chlorella stigmatophora*, attained maximal growth rates at pH 7 and 8, respectively (Fig. 2). Those two species have been reported to be highly resilient to environmental variability, rapidly growing, and living in marine polluted waters (Butcher 1952, Fabregas 1986, Cho et al. 2007). Of economic interests for industries, *Nannochloris*-like species (Henley et al. 2004) show a high potential for removing nitrogen and phosphorus from nutrient-polluted waters whilst maintaining a stable biochemical profile under environmental changes (von Alvensleben et al. 2013). Our results suggest that adding CO$_2$ to control pH is a viable tool for favouring the development of one specific microalgal species among a natural assemblage.
Figure 1. The diversity of microalgae was determined using 18S rRNA gene analysis. The first experiment ran from 17 May to 22 June 2016 (A-D). Organisms identified in the beginning of the experiment (A) and those identified at the end of the experiment in HRAPs at pH 6 (B), pH 7 (C) and pH 8 (D). The second experiment ran from 27 June to 26 July 2016 (E-H). Organisms identified at the beginning of the experiment (E) and at the end of the experiment in HRAPs at pH 6 (F), pH 7 (G) and pH 8 (H).

Although differences in microalgal diversity were observed in HRAPs, no significant differences were found among pH treatments for the photosynthetic parameters, Pmax and Ik (Table S1). However, photoinhibitory effect was significantly higher in HRAPs at pH 7 and 8 than in HRAPs at pH 6 (ANOVA p < 0.05, Tukey post hoc; Table S1). As expected, microalgae in all HRAPs were acclimated to higher Ik values and lower w values in July than in June during the experiments (ANOVA p < 0.05, Tukey post hoc; Suppl. Table S1).
Figure 2. Size fractions of algal biomass measured in HRAPs at pH 6, 7 and 8 during the first experimental run 1 (A) and the second experimental run 2 (B).

Interestingly, rhizopod development in amoebae was observed in HRAPs at pH 6 (Table 1). Rhizopod amoebae are known to feed on organic matter, bacteria, and small algal cells (Carney and Lane 2014; Day et al. 2017); they also have higher growth rates at pH lower than seawater (Rodriguez-Zaragoza 1994). The rapid development of amoebae would prevent the
abundance of small algal cells observed under the other pH conditions. In contrast, ciliates (both attached and free) grew rapidly at pH 8 and dominated the community of predators in HRAPs. Copepods (adults and nauplii) were also observed in HRAPs at pH 7 and 8 but were not observed at pH 6. The pH, therefore, seems to have a direct impact on the presence of predators of algae. Low pH has a negative effect on ciliates and copepods but a positive effect on the presence of amoebae. As a consequence, low levels of microalgal accumulation were observed at pH 6. The development of the typical marine trophic food web (ciliates, copepods) was observed in the HRAP at pH 8, leading to the dominance of very small algal cells. At intermediate pH of 7, the abundance of predators was the lowest (Table 1) and the accumulation of microalgal biomass was affected by the initial community structure of predators and temperature. Indeed, a lower level of algal biomass accumulation was observed in the HRAP at pH 7 during run 2 when the initial presence of rhizopod amoebae (Table 1) and water temperature (average level was > 25°C) was significantly higher (p < 0.05, which was determined using a t-test).

Table 1: Abundance of all the main groups of protozoans and metazoans identified by microscopy in the HRAP at pH 6, 7 and 8 at the beginning and at the end of two experimental runs of the study.

<table>
<thead>
<tr>
<th>Size fraction</th>
<th>Group (ind. L⁻¹)</th>
<th>20th of May 2016 (T0)</th>
<th>22nd of June 2016 (Tf)</th>
<th>28th of June 2016 (Tf)</th>
<th>26th of July 2016 (Tf)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pH 6</td>
<td>pH 7</td>
<td>pH 8</td>
<td>pH 6</td>
</tr>
<tr>
<td>Free ciliates</td>
<td></td>
<td>208</td>
<td>312</td>
<td>3952</td>
<td>103</td>
</tr>
<tr>
<td>Attached ciliates</td>
<td>52</td>
<td>3432</td>
<td>832</td>
<td>120016</td>
<td>11</td>
</tr>
<tr>
<td>Rhizopods</td>
<td></td>
<td>18408</td>
<td>624</td>
<td>1040</td>
<td>5</td>
</tr>
<tr>
<td>Actinopods</td>
<td></td>
<td></td>
<td></td>
<td>416</td>
<td>104</td>
</tr>
<tr>
<td>Nematods</td>
<td></td>
<td>10</td>
<td>520</td>
<td>936</td>
<td>416</td>
</tr>
<tr>
<td>Rotifers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>104</td>
</tr>
<tr>
<td>Copepods</td>
<td></td>
<td>1664</td>
<td>104</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nematods larvae</td>
<td>9</td>
<td>104</td>
<td></td>
<td>351</td>
<td>5</td>
</tr>
<tr>
<td>Tisbe sp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tisbe nauplius</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copepod nauplii</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Injection of CO₂ into HRAPs negatively affects zooplankton (Cladoceans, copepods, rotifers) through asphyxiation, allowing an increase in algal productivity and biomass (Montemezzani...
et al. 2017a, b). Similarly, in this study, no copepods grew in HRAPs where a continuous injection of CO$_2$ was done in order to maintain a pH of 6. In addition, the final abundance of protozoans and metazoans >100 µm decreased as injected CO$_2$ accumulated (lower pH) in the HRAPs (Table 1). This highlights the difficulty that large-sized aquatic organisms had maintaining a costly mode of respiration in media where CO$_2$ replaced O$_2$ availability. In contrast, the reductions in pH through the CO$_2$ injection favoured the development of rhizopods (mainly amoebae) that strongly limited algal biomass accumulation in the HRAPs. This is the first report of this observation in the literature, and it may have dramatic consequences on marine microalgae cultures in large-scale facilities.

### 3.2. Influence of pH on algal biomass accumulation

In this study, the highest accumulation of carbon as algal biomass (> 150 mg L$^{-1}$) was observed in HRAPs at pH 8 and the lowest values (< 50 mg L$^{-1}$) were observed in HRAPs at pH 6 (Fig. 3). Interestingly, algal biomass in HRAPs at the intermediate pH 7 had more variability and was influenced by seasonality, greater for run 1 than run 2 (Fig. 3). In all HRAPs, lower algal biomass was observed during run 2. Initial algal diversity was different between runs with abundance of *Nannochloris* sp. observed in June relatively to July. Water temperature was higher in July (average of 25.3°C) than in June (average of 21.8°C) and influenced predator development (Table 1), leading to higher predatory pressure on microalgae.
The loss of carbon that was photosynthetically fixed but not ultimately accumulated into algal carbon biomass was estimated from the difference between the total photosynthetic CO$_2$ uptake and the algal carbon biomass accumulated over time (Table 2). When differences were larger, algal biomass lost greater amounts of carbon through algal CO$_2$ respiration, algal carbon exudation and algal mortality by predation or viral lysis.

Table 2. Gross photosynthetic CO$_2$ uptake, produced carbon biomass and carbon losses (the fraction of the gross photosynthetic CO$_2$ uptake that was not accumulated into carbon biomass) measured in HRAPs at pH 6, 7 and 8 during the two experimental runs.
In all HRAPs, carbon loss increased rapidly over time, reaching more than 90% of the gross photosynthetic CO$_2$ uptake measured throughout the experimental period (Table 2). Most CO$_2$ loss is related to high microalgal respiration rate which has been reported to be 60-90% of the photosynthetic fixed CO$_2$ (Herzig and Falkowski 1989). Carbon loss through respiration is combined with the carbon loss through algal exudation and predation increasing with substrate limitation and time, respectively. Interestingly, the lowest carbon loss (60 and 68%) was observed within the first week of the experiments at pH 7 (Table 2). This supports our observations that intermediate pH reduces grazing on marine microalgal growth as a result of partial asphyxiation of ciliates and copepods, but maintains optimal conditions for development of amoebae that prefer more acidic waters.

3.3 Predictions of maximal biological CO$_2$ conversion efficiency in HRAP

We predicted the theoretical level of minimal carbon loss through biological activity (respiration, exudation, predation) using a GAM model with a smooth function of time and four degrees of freedom. Biological activity explained 77% of deviance ($R^2 = 0.64$) for biological carbon loss. Using this model, a new time series and confidence interval were predicted for time points that varied from 0 to 44 days using a step of 1 day (Fig. 3). The minimal fraction of gross photosynthetically fixed CO$_2$ that was not accumulated into biomass was predicted using the value reached at day 0 (Figure 4). This fraction (44 ± 25%) is considered the minimal loss of carbon due to CO$_2$ respiration required for algal metabolism (typically 20–30% of the maximal algal growth rate; Geider and Osborne 1989) and algal carbon exudation, or natural phytoplankton (7–50% of total photosynthetically fixed CO$_2$; Fogg et al. 1965).
Figure 4. The fraction (%) of gross photosynthetically fixed CO$_2$ that was not accumulated into the C biomass (biological losses) measured in HRAPs at pH 7 during run 1 and run 2. The time series was modelled using a generalised additive model (GAM) and was plotted (full lines) along with their confidence intervals (grey areas) at time points 0 to 44 days using 1-day intervals.

Therefore, the maximal theoretical CO$_2$ utilisation efficiency would be 56%, corresponding to lowest biological CO$_2$ loss (Fig.4) in an open raceway using natural marine inoculate. The maximal CO$_2$ utilisation efficiency reported during this study (40%) was measured one week after the beginning of the experiment. This value corresponds to values (31–49%) determined for CO$_2$ utilisation efficiency reported for *Nannochloris* sp. cultivated in batch mode indoors in a 10 m$^2$ raceway at pH 7.5 for 14 days (Asadollahzadeh et al. 2014). Similarly, the highest CO$_2$ utilisation efficiency observed by Asadollahzadeh et al. (2014) occurred one week after the beginning of the experiment when algal productivity was at its highest level. From our results, we show a positive correlation between water resident time and loss of fixed CO$_2$ through biological activity, especially through predation. We suggest
that microalgal cultivation time should be sufficiently short to avoid the substantial
development of predators while maintaining high algal productivity. The present experiment
was run under batch culture mode where microorganisms are inoculated to a fixed volume of
medium and they gradually consumed nutrients until exhaustion. A continuous culture mode
seems more adapted than batch culture mode, because fresh medium is continuously added to
the culture while used medium and cells are harvested at the same time. For continuous
culture mode, the dilution rate (inverse of the resident time of growing cells) can be adjusted
according to climatic variations for maintaining algal cells at high productivity rates and for
removing slow-growth predators. Our results also show that CO₂ injection can be used for
fixing pH at requested values to control the development of algal predators and to favour
rapid-growth stress-tolerant microalgae species such as *Nannochloris* sp. (Henley et al. 2002,
Vasseur et al. 2012). This supports previous observations reported in studies with CO₂
addition in HRAPs and performed in various geographical areas (New Zealand, Southern
France, Southern Spain) using natural biological inoculates (de Godos et al. 2016; Mehrabadi
et al. 2017; Uggetti et al. 2018). This suggests a common response of different natural-
inoculate biological communities to CO₂ injection in HRAPs. The use of artificial algal
assemblages, well-adapted to CO₂ capture under low pH (Piiparen et al. 2018), may facilitate
experimental procedures and industrialisation. However, a very recent study shows that a
natural algal polyculture was more productive and stress resilient than an optimised
artificially assembled polyculture when cultivated with anaerobic digester effluent and with or
not grazer additions (Thomas et al. 2019). Therefore, assembled polycultures may not be as
resilient as natural assemblages to cope with grazing pressure, variable growth conditions and
potential contaminants when using industrial flue-gas and/or wastewater
4. Conclusion

Injection of CO$_2$ for microalgae production in HRAPs requires pH control. Our results show that pH 7, controlled by CO$_2$ addition with a variability of 0.2 pH unit, favoured the growth of *Nannochloris* sp., reduced populations of marine algal predators and photosynthetically fixed accumulated CO$_2$ at 40% efficiency into carbon biomass. More acidic pH values led to the development of amoebae and restrict algal biomass accumulation. By extrapolating our experimental results, we predict that the maximal CO$_2$ conversion efficiency in these systems would be 60% of the available CO$_2$. Such value may be achievable if running HRAPs in a continuous mode, with a water resident time required for maintaining a rapid algal growth whilst reducing the development of algal predators. When using an industrial CO$_2$ source, pH regulation using injection of industrial fumes could be an effective tool for driving an algal polyculture toward more desired species. Further investigations should assess the impact of flue gas-associated contaminants on microalgal diversity, biomass production and resilience, as well as the effects of seasonal variability throughout the year on annual capability of fixing CO$_2$ and algal species selection using pH control in HRAPs.

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References


Figure S1. Values of pH measured in the HRAPs with pH targeted at 6, 7 and 8 during run 1 (from 17 May to 22 June 2016) and run 2 (from 27 June to 26 July 2016).