Journal of CO2 Utilization
May 2020, Volume 38, Pages 187-193
https://doi.org/10.1016/j.jcou.2020.01.019
https://archimer.ifremer.fr/doc/00612/72403/

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

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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 CO2 into carbon biomass (40 %) was reached at pH 7, an intermediate level, due to the partial CO2 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 CO2 into carbon biomass was estimated to be 60 % in naturally inoculated open ponds.

Highlights

► The highest conversion of CO_2 into carbon biomass was reached at pH 7. ► The theoretical maximal biological conversion of CO_2 was estimated to be *ca.* 60 %. ► Lower and higher pH favoured respectively the amoebae and copepod development. ► pH can be a tool for favouring one microalgal species among a natural assemblage.

Keywords : Carbon yield, Microalgal diversity, pH, Predators

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 gowth 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. 2017a, b, c). 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

2.2.1 Meteorological 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* (Chl*a*) and weekly for particulate organic carbon (POC) measurements. For Chl*a* measurements, between 3 and 800 ml samples (depending on sample loading) were filtered onto 13 mm GF/F fibreglassfibre filters to determine the total Chl*a* concentrations. For size-fractionated Chl*a* 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 Chla: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 ALL 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'-CTTTCCCTAACGACGCTCTTCCGATCTGCGGTAATTCCAGCTCCAA-3' and 5'-GGAGTTCAGACGTGTGCTCTTCCGATCTTTGGCAAATGCTTTCGC-3', 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 CO_2 uptake

The photosynthetic CO_2 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 (Chl*a*) was measured every day when possible in the HRAPs. Average light intensity received by microalgae was measured every 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 $m^{-2}s^{-1}$ 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 photosynthetron 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 *a* 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*a* 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*a* 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.

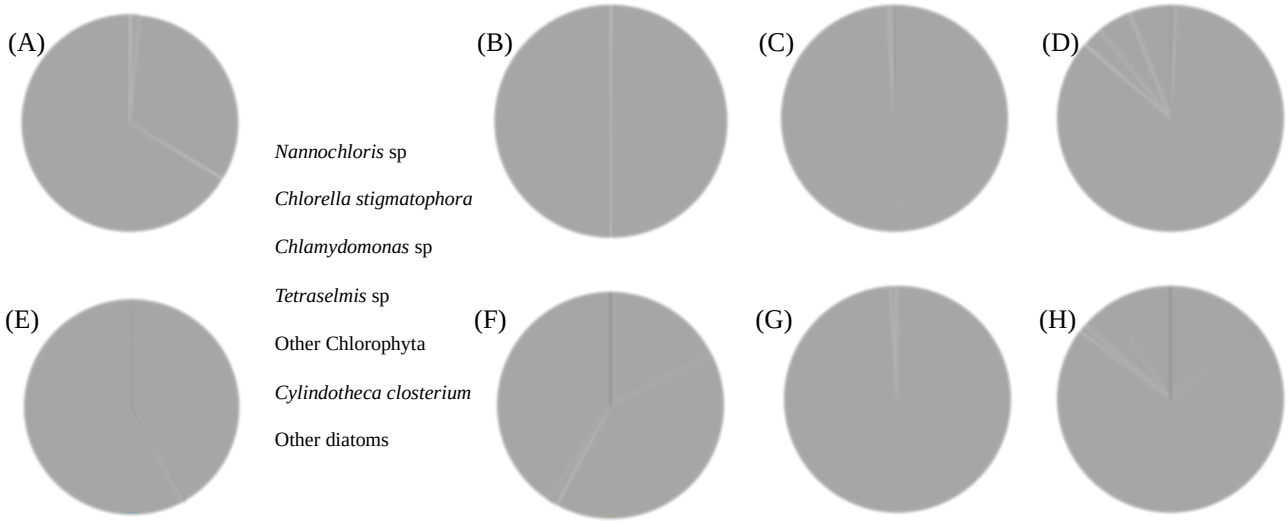

Figure 1. The diversity of microalgae was determined using 18S rRNA gene analysis. The first experiment ran from 17 May to 22 June 2016 (A-D). Organisms identified in the beginning of the experiment (A) and those identified at the end of the experiment in HRAPs at pH 6 (B), pH 7 (C) and pH 8 (D). The second experiment ran from 27 June 27 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).

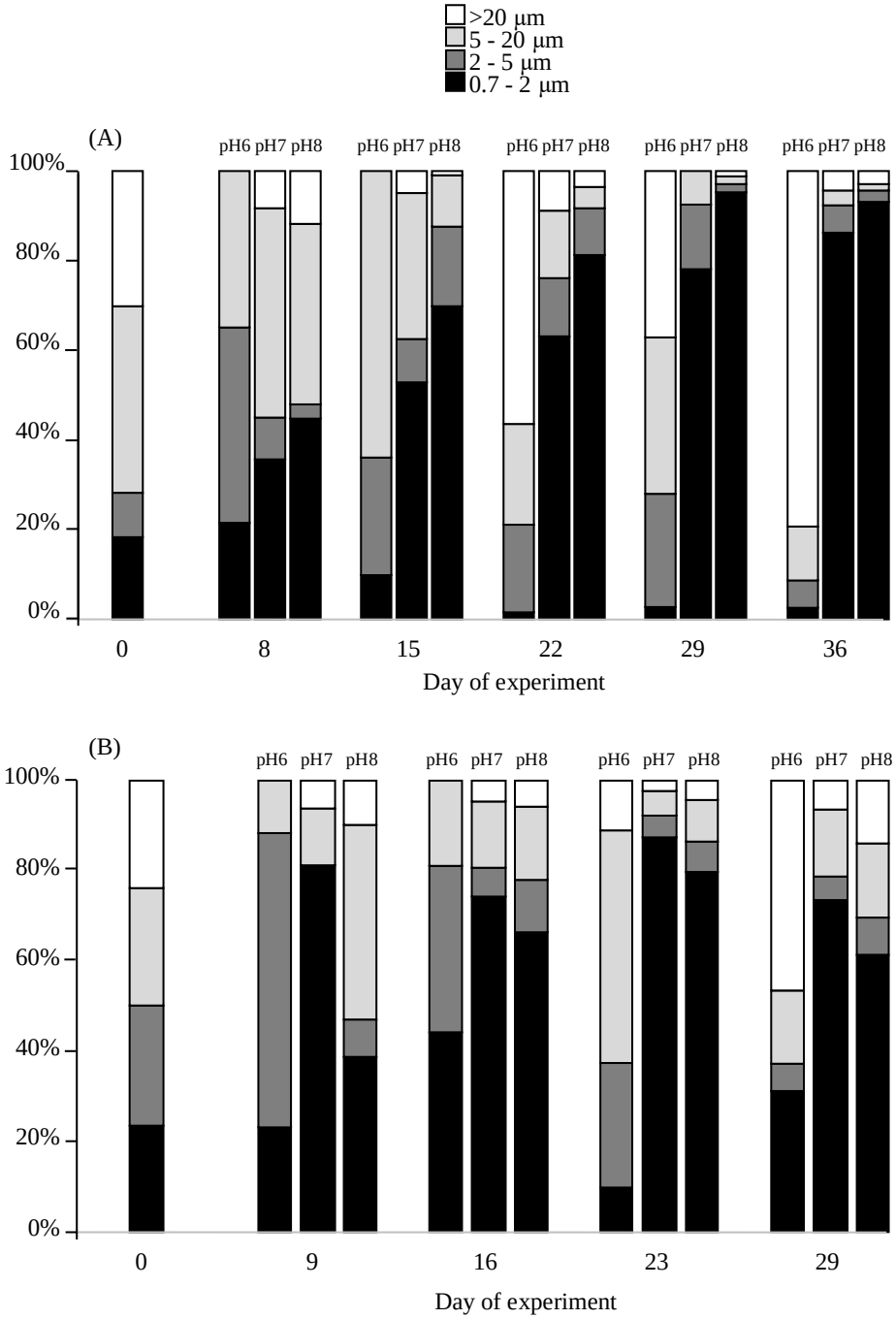

Figure 2. Size fractions of algal biomass measured in HRAPs at pH 6, 7 and 8 during the first experimental run 1 (A) and the second experimental run 2 (B).

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.

Size fraction	Group (ind. L^{-1})	20th of May 2016 (T0)	22rd of June 2016 (Tf)			28th of June 2016 (T0)	26th of July 2016 (Tf)		
			pH 6	pH 7	pH 8		pH 6	pH 7	pH 8
35-100 mm	Free ciliates	52	208	312	3952	103	3328	1664	34320
	Attached ciliates		3432	832	120016			1040	43888
	Rhizopods		18408	624	1040	11	57928	8008	1352
	Actinopods				416		1976	416	208
	Nematods	10	520	936	416		104	2184	
	Rotifers								520
	Copepods			1664	104			312	2600
> 100 mm	Nematods larvae	9	104		351	5	50	513	169
	Tisbe sp.			299	156				871
	Tisbe nauplius	0							104
	Copepod nauplii			59					

Injection of CO_2 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 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.

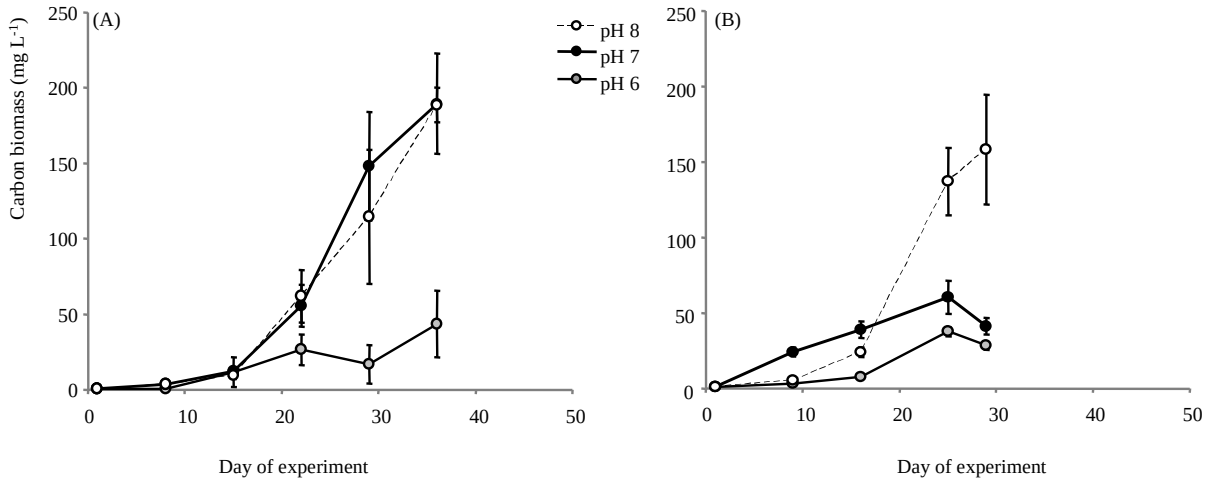

Figure 3. Concentration of carbon biomass (POC measurements) measured in HRAPs (average and standard deviation of sample triplicates) during run 1 from 17 May to 22 June 2016 (A) and during run 2 from 27 June to 26 July 2016 (B).

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.

Date	Day of experiment	pH 6			pH 7			pH 8		
		Gross photosynthetic CO_2 uptake (mgC L^{-1})	Produced C biomass (mgC L^{-1})	C loss (%)	Gross photosynthetic CO_2 uptake (mgC L^{-1})	Produced C biomass (mgC L^{-1})	C loss (%)	Gross photosynthetic CO_2 uptake (mgC L^{-1})	Produced C biomass (mgC L^{-1})	C loss (%)
20/05/16	3	0	0.0		0	0		0	0	
25/05/16	8	10	0.1	99	10	3	68	19	3	85
01/06/16	15	600	11	98	159	12	93	147	9	94
08/06/16	22	2270	26	99	767	55	93	1079	61	94
15/06/16	29	2328	16	99	1431	148	90	1985	114	94
22/06/16	36	2541	43	98	2602	189	93	3760	188	95
28/06/16	1	0	0.0		0	0		0	0	
06/07/16	9	22	2.1	91	57	23	60	15	4	73
13/07/16	16	371	6.2	98	386	38	90	203	23	89
22/07/16	25	877	36	96	875	59	93	1246	136	89
26/07/16	29	1074	27	97	1180	40	97	1620	157	90

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).

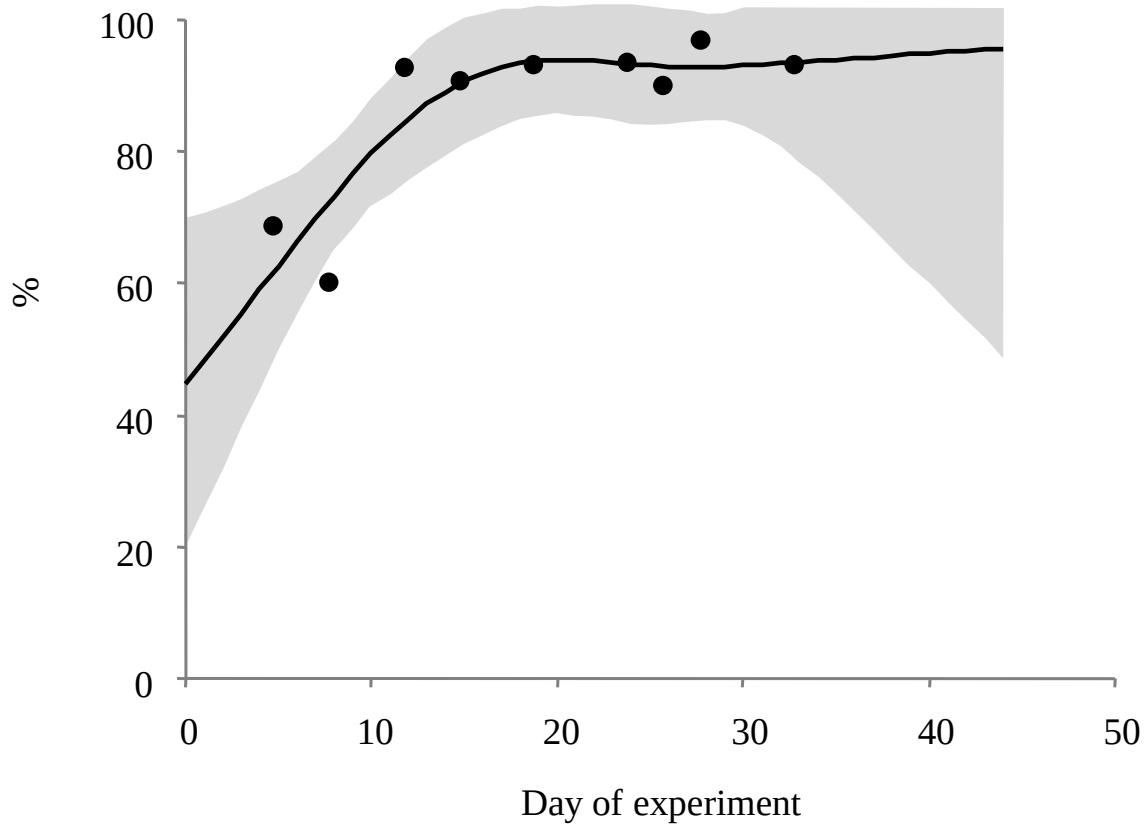

Figure 4. The fraction (%) of gross photosynthetically fixed CO_2 that was not accumulated into the C biomass (biological losses) measured in HRAPs at pH 7 during run 1 and run 2. The time series was modelled using a generalised additive model (GAM) and was plotted (full lines) along with their confidence 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 CO_2 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 CO_2 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 CO_2 injection in HRAPs. The use of artificial algal assemblages, well-adapted to CO_2 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.

Acknowledgments

This study was supported by VASCO2 project, which was funded by the Agence de l'Environnement et le la Maîtrise de l'Energie – French Environment and Energy Management Agency (ADEME). We would like to thank Patrick Raimbault (UMR MIO, Marseille) for analysing the carbon content of biomass.

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Supplementary material

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).

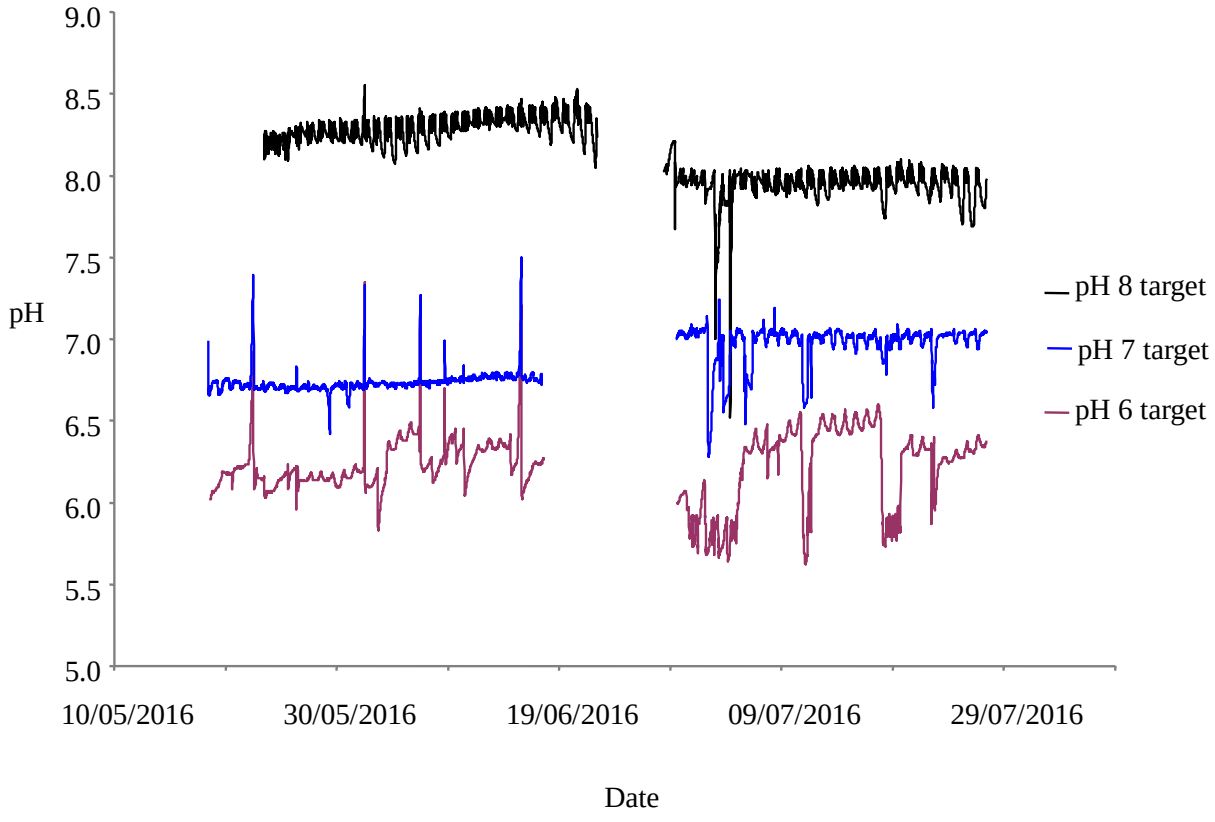

Table S1. Photosynthetic parameters measured weekly in each HRAP at pH 6, 7 and 8 throughout two runs of the study.

Date	Day of experiment	pH 6			pH 7			pH 8		
		Pmax	Ik	w	Pmax	Ik	w	Pmax	Ik	w
25/05/16	8	14	3	260	5.6	14	4.7	10	48	16
01/06/16	15	26	51	14	19	22	2.0	23	69	10
08/06/16	22	1.0	1	49	6.8	18	45	8.5	37	21
15/06/16	29	2.8	11	78	3.7	10	68	6.6	52	14
22/06/16	36	1.6	7	120	2.8	26	31	1.6	20	22
28/06/16	1	7.3	1	70	ND	ND	ND	ND	ND	ND
06/07/16	9	18.0	161	3.4	11	123	4.9	12	96	7.3
13/07/16	16	11.3	26	28	9.1	111	0.02	8.0	108	6.1
22/07/16	25	6.2	25	31	6.9	106	0.8	4.4	121	1.7
26/07/16	29	8.8	38	21	5.0	25	32	2.7	15	20

Pmax is the maximal production rate (mg O_2 mg Chla^{-1} h^{-1}), Ik is the light saturation intensity (µmol m^2 s^{-1}) and w is an indicator of the relative magnitude of photoinhibition derived from the relationship between light intensity and the rate of photosynthesis (Eilers and Peeters 1988)