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Report on literature review on algae growth in extreme pH

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Report Title: Report on literature review on algae growth in extreme pH

Key words: microalgae, CO_2 uptake, growth, pH, acidic, alkaline

Abstract

Microalgae are typically grown at pH values between 6.5 and 8, but CO_2 provision may be more efficient at higher or lower pH. D601 (Review of concepts and technologies for capturing CO2 by algae, S. Teir) of this program concluded that bubbling carbonation columns provide the most promising system for CO_2 provision to algal systems, which would optimally include alkali salts that could be recycled in the sytem. The pH at which such columns would operate would be determined by the characteristics of the algal species feeding on the captured CO_2 (as bicarbonate). On the other hand, direct input of CO_2-rich gas is often used to mix algal cultures and could be carried out at low pH values. In this review we identified species that grow at high or low pH values, with an emphasis on species for which some parameter of growth had been measured. *Euglena*, *Coccomyxa* and *Chlorella* species have been observed to grow at low pH values (pH < 3), often associated with envrionments containing heavy metals. Slower growing species included *Dunaliella*, *Cyanidium* and *Galdieria*. Few cyanobacteria have been reported to grow at low pH values, but growth of *Scenedesmus acutus* was reported at pH 4. Species of various diatoms, including *Thalassiosira*, *Fragilariopsis*, and *Phaeodactylum*, as well as several cyanobacteria and green algae in the *Chlamydomonas* and *Neochloris* genera are reported to grow at pH values of 9 or higher. Specific growth rates of alkali-tolerant species were generally low at pH values above 9.5. Based on reported growth rates, there is no clear advantage for algal growth in providing CO_2 as bicarbonate, rather than as gaseous CO_2, or vice-versa.

Espoo, August 2016

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1 Introduction

The group of organisms generally referred to as algae, including microscopic, single cell organisms, macroscopic "seaweeds" and often also "blue-green algae" or cyanobacteria, are responsible for at least half of the world's photosynthesis. During photosynthesis, CO_2 is taken into a cell, either as CO_2 or as bicarbonate, the carbon is converted into biomass or other products and oxygen is released to the atmosphere. The potential for algal capture of CO_2 is generally agreed to be higher than that of land plants, at least for those species which have high growth rates (Chisti, 2007; Darzins et al., 2010). Many species grow under conditions such as high salinity, which are not suitable for plants, adding to the interest in algal cultivation. While much current algal research focuses on the potential of various species as sources of biofuel, other bulk products, nutriceuticals and pharmaceuticals, algal growth may also be considered primarily from the perspective of CO_2 capture. CO_2 is captured and sequestered as algal biomass, from which it may be released relatively rapidly (e.g. if the biomass is used as fuel or animal feed) or more slowly (e.g. if biomass is used as fertiliser). Optimally CO_2 would be captured from industrial sources of flue gas. Flue gas may be directly injected into an algal culture system to provide mixing as well as CO_2 to the system. Direct injection lowers the pH of aqueous systems, for which low pH tolerant organisms could be beneficial. Alternatively, CO_2 from flue gas may be captured as carbonate or bicarbonate using an aqueous or chemical scrubber (Canon-Rubio et al., 2016; Chi et al., 2011) and is then fed to the algae as an aqueous solution. High pH values are needed to ensure that the bicarbonate remains in solution and is not released to the atmosphere. Alkaline tolerant algae are desirable when CO_2 is fed as bicarbonate/carbonate. D601 (Review of concepts and technologies for capturing CO2 by algae, S. Teir) of this program concluded that bubbling carbonation columns provide the most promising system for CO_2 provision to algal systems, which would optimally include alkali salts that could be recycled in the sytem.

Microalgae are typically grown at pH values between 6.5 and 8. Species which tolerate much lower or higher pH values have been identified, e.g. from acidic rivers, oceans and soda lakes, and some of these have been characterised. The objective of this review was to establish which algae grow at the two pH extremes, to collect data on their growth rates (specific or linear) as a measure of ability to capture CO_2, and if available information on CO_2 uptake in acidic or alkali conditions. Literature concerning growth of algae at pH values between 5 and 8 is not included in this review, but data within this range is included when it was available in publications referring to growth at pH values less than 5 and greater than 8. Direct comparison of growth rates between species is not possible, since the methods used to obtain the data differs, but the relative data provides insight into the question of whether growth at either high or low pH would inherently have better potential for CO_2 capture.

2 Microalgal growth at low pH

Algae which are tolerant to and grow at low pH values have generally been studied in respect to environmental problems, such as heavy metal contamination in acidic rivers affected by local mining (Nalewajko et al., 1997; Niyogi et al., 1999; Olaveson and Nalewajko, 2000) or low pH soils (Gopalaswamy et al., 2007).

Table 1 lists species which have been shown to grow at low pH values. Specific growth rate was the most consistently measured parameter, although some articles also measured the linear growth phase, the biomass concentration or a measure of photosynthesis. Units of measure of photosynthesis, quantified as O_2 evolution or CO_2 incorporation, differed widely and could not be used to compare the CO_2 uptake potential of organisms reported in different publications. Direct measurement of CO_2 uptake for algae growing at extreme pH was only reported in two publications, both of which used radioactive carbon to determine CO_2 uptake, feeding air and $^{14}CO_2$ (Gyure et al., 1987) or $NaH^{14}CO_3$ (Rai et al., 1993). Several articles report only the identity of algae which are found in their study of extreme pH, without providing any measurement of growth or photosynthesis (Table 1). For example, species of *Klebsormidium*, *Dunaliella* and *Cyanidium* have been isolated from an acidic (pH 0.9) part of the Rio Tinto River (Aguilera, 2013), but their growth under this condition has not been charactererised.

*Table 1. Species reported to grow at low pH values and their growth or photosynthetic characteristics. * heavy metals present. – = not determined. Chla = chlorophyl a*

Specie	**pH**	**μ (day^{-1})**	**μ (g day^{-1}, g L^{-1} d^{-1})**	**Biomass (g L^{-1})**	**CO_2 feed**	**Photosynthesis**	**Reference**
*Klebsormidium**	0.9	-	-	-	-	-	Aguilera, 2013
*Dunaliella**	0.9	-	-	-	-	-	Aguilera, 2013
*Cyanidium**	0.9	-	-	-	-	-	Aguilera, 2013
*Chlorella**	1.6					0.14 mg O_2 mg $Chla^{-1}$ h^{-1}	Aguilera, 2013; Souza-Egipsy et al., 2011
*Pinnularia**	1.8	-	-	-	-	1.42 mg O_2 mg $Chla^{-1}$ h^{-1}	Aguilera, 2013; Souza-Egipsy et al., 2011
*Euglena**	1.8					0.26 mg O_2 mg $Chla^{-1}$ h^{-1}	Aguilera, 2013; Souza-Egipsy et al., 2011
Galdieria sulphuraria	2	0.1	-	-	-	-	Sloth et al., 2006
*Euglena gracilis**	2	0.328	-	-	-	-	Olaveson and Nalewajko, 2000
*Euglena mutabilis**	2	0.387	-	-	-	-	Olaveson and Nalewajko, 2000
Algae + bacteria	2	-	-	-	-	11000 cpm $^{14}CO_2$	Gyure et al., 1987

Specie	pH	μ (day^{-1})	μ (g day^{-1}, g L^{-1} d^{-1})	Biomass (g L^{-1})	CO_2 feed	Photosynthesis	Reference
*Chlorella**	2.4	-	-	-	-	0.45 mg O_2 mg Chla^{-1} h^{-1}	Aguilera, 2013; Souza-Egipsy et al., 2011
*Euglena**	2.4					0.28 mg O_2 mg Chla^{-1} h^{-1}	Aguilera, 2013; Souza-Egipsy et al., 2011
Coccomyxa onubensis	2.5	0.163[a], 0.170, 0.190[b], 0.233[c] 0.236 0.300[d] 0.413[c] 0.698[c]	- - - - 0.41 - 0.25 0.19	0.5 0.5 1.0 - 0.5 0.5 0.16	5 % 0.03 % 5 % 5 % 5 % 5 % 5 % 5 %	-	Vaquero et al., 2014a; Vaquero et al., 2014b
*Euglena mutabilis**	2.5	0.774	-	-	-	-	Olaveson and Nalewajko, 2000
*Euglena gracilis**	2.5	1.072	-	-	-	-	Olaveson and Nalewajko, 2000
*Euglena**	2.5	-	-	-	-	0.45 to 0.56 mg O_2 mg Chla^{-1} h^{-1}	Aguilera, 2013; Souza-Egipsy et al., 2011
Algae + bacteria	2.6	-	-	-	-	20000 cpm $^{14}CO_2$	Gyure et al., 1987
*Zygnemopsis**	2.8	-	-	-	-	0.25 mg O_2 mg Chla^{-1} h^{-1}	Aguilera, 2013; Souza-Egipsy et al., 2011
Chlorella kessleri	3	0	-	-	-	-	El-Ansari & Colman 2015
*Euglena mutabilis**	3	0.834	-	-	-	-	Olaveson and Nalewajko, 2000
*Euglena gracilis**	3	1.043	-	-	-	-	Olaveson and Nalewajko, 2000
Algae + bacteria	3	-	-	-	-	16000 cpm $^{14}CO_2$	Gyure et al., 1987
Chlorella vulgaris mutant	3.5	-	-	-	-	36 mg O_2 μg protein^{-1} h^{-1} 2921 cpm $^{14}CO_2$ μg protein^{-1} **	Rai et al., 1993
Chlorella kessleri	4	0.221	-	-	-	9 nmol O_2 (ml cell)$^{-1}$ min^{-1}	El-Ansari and Colman, 2015
Coccomyxa onubensis	4	0.255	0.42	-	5 %	-	Vaquero et al., 2014a
*Euglena mutabilis**	4	0.834	-	-	-	-	Olaveson and Nalewajko, 2000
*Euglena gracilis**	4	1.087	-	-	-	-	Olaveson and Nalewajko, 2000
*Scenedesmus acutus**	4	-	-	-	-	50 μmol O_2 (10^9 cell)$^{-1}$ h^{-1}	Nalewajko et al., 1997
Chaetomorpha macroalga	4	-	-	-	-	3 mg O_2 g DW^{-1} h^{-1}	Menéndez et al., 2001
Chlorella vulgaris mutant	4	-	-	-	-	44 mg O_2 μg protein^{-1} h^{-1}	Rai et al., 1993

Specie	pH	μ (day^{-1})	μ (g day^{-1}, g L^{-1} d^{-1})	Biomass (g L^{-1})	CO_2 feed	Photosynthesis	Reference
						3166 cpm $^{14}CO_2$ μg protein^{-1} **	
*Scenedesmus acutus**	4.5	0.304 [e]	-	-	-		Nalewajko et al., 1997
Chlorella kessleri	4.5	-	-	-	-	10 nmol O_2 (ml cell)$^{-1}$ h^{-1}	El-Ansari and Colman, 2015
*Scenedesmus acutus**	4.8	0.416 [e]	-	-	-		Nalewajko et al., 1997
SM0708 cyanobacterium	4.9	0.749 to 1.6	-	-	-	-	Jasser et al., 2013
KS0708 cyanobacterium	4.9	0.94 to 2.2	-	-	-	-	Jasser et al., 2013
*Scenedesmus acutus**	5	0.448[e]	-	-	-	75 μmol O_2 (10^9 cell)$^{-1}$ h^{-1}	Nalewajko et al., 1997
Chlorella kessleri	5	0.519	-	-	-	11 nmol O_2 (ml cell)$^{-1}$ min^{-1}	El-Ansari and Colman, 2015
*Euglena mutabilis**	5	0.834	-	-	-	-	Olaveson and Nalewajko, 2000
*Euglena gracilis**	5	1.028	-	-	-	-	Olaveson and Nalewajko, 2000
Chlorella vulgaris	5	-	-	-	-	45 mg O_2 μg protein^{-1} h^{-1}; 3348 cpm $^{14}CO_2$ μg protein^{-1} **	Rai et al., 1993

[a] light 50 μmol m^{-1} s^{-1}
[b] light 650 μmol m^{-1} s^{-1}
[c] light 400 μmol m^{-1} s^{-1}
[d] light 140 μmol m^{-1} s^{-1}
[e] light 130 μmol m^{-1} s^{-1}

Euglena species were prevalent among algae growing well (0.8 to 1 day^{-1}) at low pH. The specific growth rates of *Euglena* species growing at pH 3 are generally comparable to those of the same species growing in similar conditions at pH 6 (Table 1, Figure 1). A wider variety of species grow at pH values between 4 and 5 than at 3 or lower (Table 1), including some cyanobacteria (Jasser et al., 2013) and diatoms (Souza-Egipsy et al., 2011), which are more typically found among alkali tolerant species. Specific growth rates at pH values below 2.5 were generally low (0.1 to 0.4 day^{-1}) compared to those observed at pH 3 or higher (0.2 to 1 day^{-1}; Table 1), but a *Coccomyxa onubensis* was observed to grow at a specific growth rate of 0.7 day^{-1} at pH 2.5 when provided sufficient CO_2 and light (Isabel Vaquero et al., 2014).

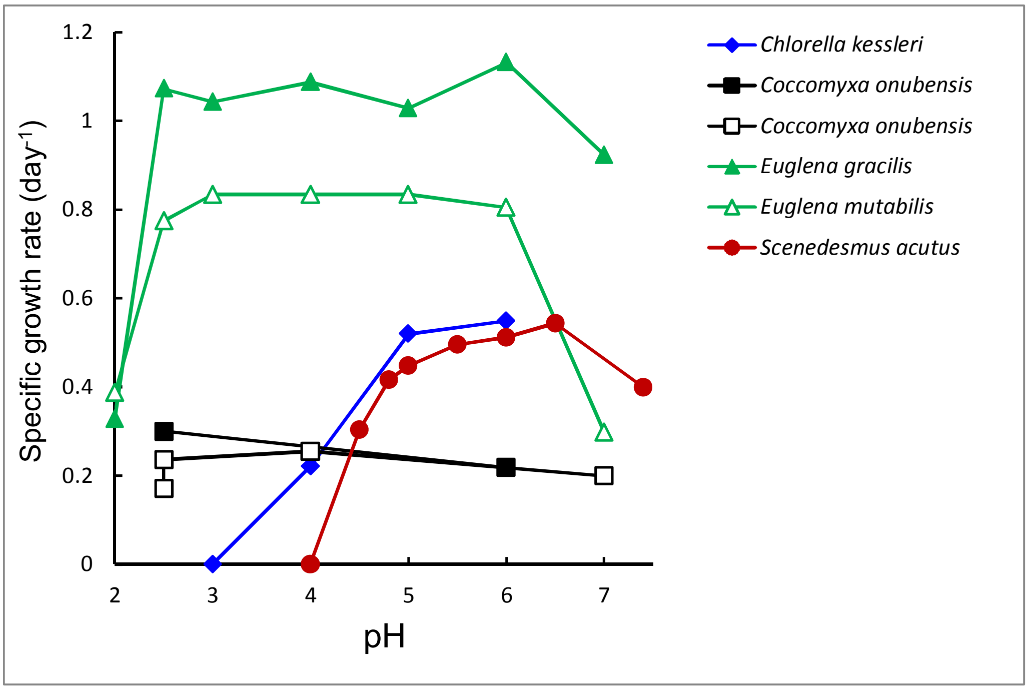

Figure 1. Specific growth rates of acid tolerant algae for which data was available at pH values between 2 and 7.5. See Table 1 for references from which the data is dervied.

3 Microalgal growth at high pH

Many of the algal species reported to grow at pH values above 8.5 are marine species, with diatoms being particularly well represented (Table 2). Specific growth rates of algae growing in alkaline conditions were more widely reported (Table 2) than of algae growing in acidic conditions (Table 1), but fewer authors reported measurements of photosynthesis. The specific growth rates of algae growing at pH values above 8 were between 0.05 and 1.8 day^{-1} (Table 2). *Thalassiosira pseudonana* and *T. oceanica* had the highest specific growth rates at high pH values (1.7 to 1.8 day^{-1} at pH ~9) of the algae for which specific growth rates have been reported. The green alga *Neochloris oleoabundans* UTEX 1185 has been grown at specific growth rates up to 1.9 day^{-1} in chemostat culture at pH 10, although biomass concentration decreased with increasing dilution rate (Santos et al., 2013). *Euhalothece* sp. ZM001, a cyanobacterium, has also been shown to grow at rates above 1 day^{-1} when provided adequate light and CO_2 (Chi et al., 2013). Other algae generally grew at rates less than 0.5 day^{-1} (Table 2, Figure 2). Both species of *Thalassiosira* grew only slightly slower at pH 9 than at pH 7, but the specific growth rate was much lower at pH values above 9 (Munir et al., 2015). Similar observations can be made for other alkalophilic algae, with a few exceptions such as *Coelastrella* sp. (Gardner et al., 2010), *Phaeodactylum tricornutum* (Spilling et al., 2013) and *Scenedesmus* sp. (Gardner et al., 2010), which showed similar growth at pH >10 as at pH ~7.5 (Figure 2). Ability to sustain high growth over a pH range of 7 to 10 is benficial when supplying CO_2 as bicarbonate using an alkali scrubber to initially capture the CO_2 from the flue gas (Canon-Rubio et al., 2016).

*Table 2. Species reported to grow at high pH values and their growth or photosynthetic characteristics. * chemostat (D = 0.2 day^{-1}). **macroalga with maximum photosynthetic rate at pH 6 to 8. – = not determined.*

Species	**pH**	**µ (day^{-1})**	**µ (g day^{-1}, g L^{-1} d^{-1})**	**Biomass (g L^{-1})**	**CO_2 feed**	**Photosynthesis**	**Reference**
Oedogonim sp.	8.5	0.054	0.15	-	-	-	Munir et al., 2015
Spirogyra sp.	8.5	0.058	0.12	-	-	-	Munir et al., 2015
*Ettlia**	8.5	0.2	0.097 0.088	0.48 0.44	10 % -	8.1 pg O_2 cell^{-1} h^{1} 7.9 pg O_2 cell^{-1} h^{1}	Yoo et al., 2015
Fragilariopsis nana	8.5	0.43	-	-	-	-	Søgaard et al., 2011
Fragilariopsis sp.	8.5	0.5	-	-	-	-	Søgaard et al., 2011
Chlamydomonas sp.	8.5	0.52	-	-	-	-	Søgaard et al., 2011
Thalassiosira oceanica	8.5	2.2	-	-	-	-	Chen and Durbin 1994
Scenedesmus sp.	8.7	0.33	-	0.71	-	-	Gardner et al., 2010
Ceratium lineatum	8.8	0.05	-	-	-	-	Hansen, 2002
Prorocentrum minimum	8.8	0.45	-	-	-	-	Hansen, 2002
Spirogyra sp.	9	0.054	0.09	-	-	-	Munir et al., 2015
Oedogonim sp.	9	0.058	0.12	-	-	-	Munir et al., 2015
Coccomyxa onubensis	9	0.182	0.27	-	5 %	-	Vaquero et al., 2014a
Fragilariopsis sp.	9	0.32	-	-	-	-	Søgaard et al., 2011
Fragilariopsis nana	9	0.39	-	-	-	-	Søgaard et al., 2011
Chlamydomonas sp.	9	0.41	-	-	-	-	Søgaard et al., 2011
Chlorella kessleri	9	0.479	-	-	-	-	El-Ansari and Colman, 2015
Thalassiosira pseudonana	9	1.8	-	-	-	-	Chen and Durbin, 1994
*Chaetomorpha***	9	-	-	-	-	3 mg O_2 (g DW)$^{-1}$ h^{-1}	Menéndez et al., 2001
Heterocapsa triquetra	9.1	0.58	-	-	-	-	Hansen, 2002
Thalassiosira oceanica	9.1	1.7	-	-	-	-	Chen and Durbin, 1994
Scenedesmus sp.	9.3	0.31	-	0.96	-	-	Gardner et al., 2010
Coelastrella sp.	9.3	0.38	-	1.08	-	-	Gardner et al., 2010
Thalassiosira pseudonana	9.3	0.6	-	-	-	-	Chen and Durbin, 1994
Heterocapsa triquetra	9.4	0.14	-	-	-	-	Hansen, 2002

Species	pH	μ (day^{-1})	μ (g day^{-1}, g L^{-1} d^{-1})	Biomass (g L^{-1})	CO_2 feed	Photosynthesis	Reference
Thalassiosira pseudonana	9.4	0.3	-	-	-	-	Chen and Durbin, 1994
Prorocentrum minimum	9.5	0.11	-	-	-	-	Hansen, 2002
Fragilariopsis sp.	9.5	0.23	-	-	-	-	Søgaard et al., 2011
Fragilariopsis nana	9.5	0.24	-	-	-	-	Søgaard et al., 2011
Chlamydomonas sp.	9.5	0.29	-	-	-	-	Søgaard et al., 2011
Thalassiosira oceanica	9.5	0.8	-	-	-	-	Chen and Durbin, 1994
Fragilariopsis sp.	10	0	-	-	-	-	Søgaard et al., 2011
Fragilariopsis nana	10	0	-	-	-	-	Søgaard et al., 2011
Chlamydomonas sp.	10	0.14	-	-	-	-	Søgaard et al., 2011
Phaeodactylum tricornutum	10	0.3	-	-	-	-	Spilling et al., 2013
Euhalothece ZM001	10	~1.3	-	-	1 M $NaHCO_3$	-	Chi et al., 2013
Neochloris oleoabundans	10	0.6 1.0 1.4 1.9	- - - -	0.6 0.46 0.33 0.22	+ + + +	- - - -	Santos et al., 2013
Coelastrella sp.	10.4	0.27	-	1	-	-	Gardner et al., 2010
Scenedesmus sp.	10.4	0.40	-	0.71	-	-	Gardner et al., 2010
*Ettlia**	10.5	0.2	0.087 0.066	0.435 0.33	10 % -	2.1 pg O_2 $cell^{-1}$ h^1 7.1 pg O_2 $cell^{-1}$ h^1	Yoo et al., 2015

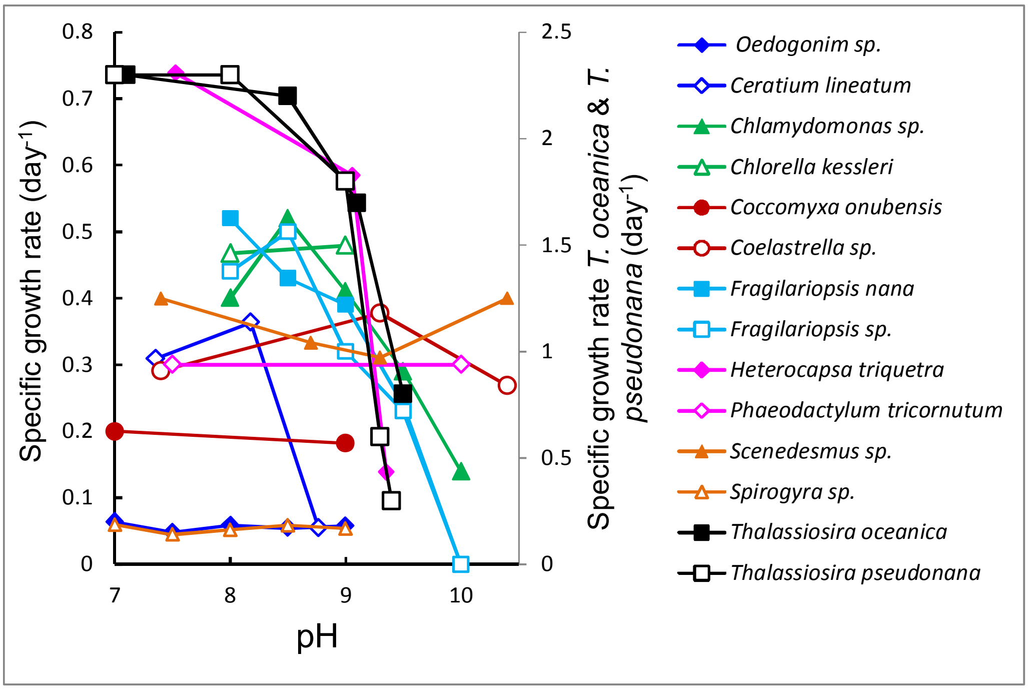

Figure 2. Specific growth rates of alkali tolerant algae at pH values between 7 and 10.5. Note that the specific growth rates of T. oceanica *and* T. pseudonana *are shown on the scale to the right, while all others are shown on the scale to the left. See Table 2 for references from which the data is derived.*

4 Conclusions

Although algae are commonly grown at pH values between 6.5 and 8, there are numerous algae which grow well in conditions of more extreme pH. Based on published data, the growth rates achieved in acidic and alkali conditions were similar, although specie-dependent (Figure 3). Three species (*T. pseudonana*, *T. oceanica*, and *N. oleoabundans*) were observed to grow at higher specific growth rates in alkaline conditions than the best algae (*E. gracilis* and *E. mutabilis*) in acidic conditions, but the range of specific growth rates of other species was similar. Assuming that specific growth rate reflects ability to take up CO_2, either as CO_2 at low pH or as either CO_2 or bicarbonate (HCO_3^-) at high pH, this suggests that there is no inherent benefit or disadvantage from the biological point of view, to providing CO_2 as gas or as dissolved bicarbonate, provided the appropriate specie(s) is used in the cultivation. In order to utilise CO_2 in alkaline conditions, the alga should have mechanisms for utilising bicarbonate (Giordano et al., 2005; Holmes-Farley, 2006). Alternatives to use of air for culture mixing should also be considered for bicarbonate-fed cultures, to avoid loss of CO_2 to the atmosphere (Rodríguez-Maroto et al., 2005).

Canon-Rubio et al. (2016) have demonstrated that the use of alkaline scrubbers for CO_2 transfer to algal cultures would be theoretically feasible for alkali-tolerant algae and Yadav et al. (2016) used an alkaline scrubber to feed CO_2 to cultures of

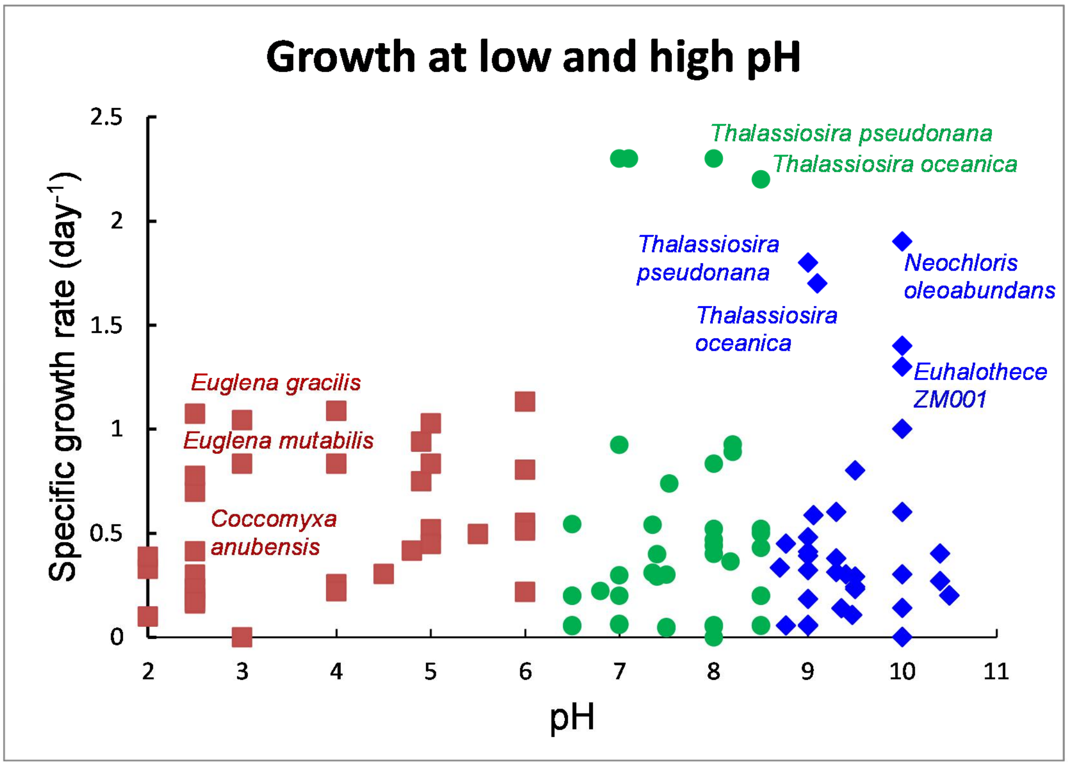

Figure 3. Published measurements of algal growth at pH values below 5 (red) or above 8.5 (blue) for various species. Data at pH values between 5 and 8.5 are also shown, for species which had tolerance to high or low pH. See Tables 1 & 2 for details of the species and the associated references.

Chlorella vulgaris in a pH range between 7 and 10. Bicarbonate has also been used as carbon source in various small scale cultivations (Chi et al., 2014). Canon-Rubio et al. (2016) recommended the use of mixed algal populations for optimal capture of CO_2 and production of biomass in alkali systems. However, it should be noted that diatoms (e.g. *T. pseudonana*, *T. oceanica*, *Euhalothece* sp., *Phaeodactylum* sp.) may not be desirable components of such consortia, in spite of their high specific growth rates and tolerance to alkaline pH, because of their requirements for silicates and vitamins which increase the cost of cultivation.

To optimise CO_2 uptake, species can be chosen based only on their capacity for CO_2 uptake, nutrient requirements and environmental constraints. However, algal CO_2 capture generates algal biomass that would generally be extracted or converted to other products. The choice of end products may impose further restrictions on the choice of specie(s) and the mode of cultivation for CO_2 capture. Several of the acid tolerant species have been used to obtain nutritional supplements and CO_2 would need to be provided to these in gaseous form. Low pH systems do not need to be as low as the extremes considered here, but use of the extremes at both ends of the pH scale will contribute to the maintenance of stable popultions with reduced opportunities for invading contaminants.

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