

Dependency of Microalgal Production on Biomass and the Relationship to Yield and Bioreactor Scale-up for Biofuels: a Statistical Analysis of 60+ Years of Algal Bioreactor Data

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Abstract Since the 1950s, research has been undertaken to promote algal oil as a sustainable alternative to fossil fuels. This paper statistically analyzed 317 studies of algal bioreactors to determine the interdependence of biological and physical factors affecting oil yield. Algal growth rates in bioreactors often (71 %) exceeded maximal growth rates cited in the literature, and biomass was generally higher than maximum values cited for laboratory cultures. Growth rate decreased with increasing biomass, and biomass, not growth, dominated production rate, which was higher in closed than in open bioreactors. Except for *Chlorella* cultured in horizontal tubular reactors, there were no statistical differences in algal production when grown in different types of reactors. Production decreased with increasing bioreactor volume, but increased with surface to volume ratio of the bioreactor. In contrast, estimated oil yields increased with bioreactor volume. Four groups of bioreactors were identified based on their oil yields and biomass production: (1) higher yields with lower production were limited to open systems with volumes $\geq 10^4$ L; (2) higher yields with higher production were almost exclusively closed bioreactors from 10^2 to 10^3 L; (3) lower yields with higher production were closed systems from 3 to 99 L; and (4) lower yields with lower production were a mix of open and closed systems with diverse volumes. Based on these groups, it is suggested that intermediate volume bioreactors with higher surface to volume ratios could give higher yields and production rates and would avoid the environmental and

scale-up problems inherent in large bioreactors currently being used commercially to culture microalgae.

Keywords Algal bioreactors · Productivity · Biomass · Scale-up

Introduction

Research on algal production rates for biofuels and commercial products has led to a plethora of work on open bioreactors, such as ponds [1–34] and raceway flumes [4, 34–66], and closed bioreactors, such as vertical [67–91], horizontal [34, 40, 92–142] and helical tubes [97, 143–154], flat plates [66, 155–184], and other unique designs [133, 185–189]. This research dates back to the early 1950s and was chronicled in a historical perspective by Borowitzka [190].

Most species that have been studied produce only 30–50 % of their biomass as lipids [191, 192]. Given this limitation in cell lipid storage, the focus on increased biofuel yields has been on operating bioreactors to optimize the culture conditions and increase production. To maintain high yields of biofuel from microalgae-based processes, it is essential to devise culture systems that deliver high lipid content and high primary productivity. The former can be achieved by selecting target species to optimize neutral lipid production and storage. The later can be achieved by increasing biomass, cell growth rate, and volume of the culture system. High biomass, growth rate, and to some extent, lipid content are enhanced by optimal culture conditions through regulation of temperature, nutrients, and irradiance. However, to achieve these culture conditions, the size of the bioreactor can vary from the laboratory flasks of 10^{-1} L to large raceway ponds, on the order of 10^6 L.

Many excellent reviews have compared the various designs and provided photographs and illustrations of these bioreactor

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systems [66, 96, 190, 193–201]. However, few papers have examined statistical relationships from these bioreactor studies, with the exception of Williams and Laurens [199], who determined that closed bioreactors were more productive than open systems. This paper has compiled over 60 years (1953–2015) of data from review articles and their original references, special reports, and recent articles on algal culture systems and has statically analyzed optimum algal production rates (i.e., algal primary production or productivity) based on the algal class, cell volume, growth rates, biomass concentration, the bioreactor type, and the volume of the bioreactor. Maximum growth rates in laboratory cultures were compared to growth rates in larger (>1 L) bioreactors. Bioreactor biomass was compared with data from both the laboratory and phytoplankton ecology. Finally, the most effective culture systems for increasing production rate were examined, as well as how predicted oil yields vary with scale-up of the culture system. This paper does not evaluate the types of lipids produced by algae, which is very important to biofuel commercialization—see “A matter of details” [202], but focuses instead on the efficacy of bioreactors as culture systems.

Methods

Data were collected from 189 articles, comprising 317 experiments on the cultivation of microalgae in open and closed bioreactors. From these articles, information was collected on algal species, specific growth rate (μ), biomass of the culture (B), production rate (P), and bioreactor type, volume (V) and illuminated surface area (SA). All articles reported production rates as either mass per volume per time ($\text{g L}^{-1} \text{day}^{-1}$ as dry weight) or mass per surface area per time ($\text{g m}^{-2} \text{day}^{-1}$). In this paper, the bioreactor volumes are in liters; therefore, units of $\text{g m}^{-2} \text{day}^{-1}$ were converted to $\text{g L}^{-1} \text{day}^{-1}$ by multiplying the appropriate surface area and dividing by the culture volume in liters. For studies that did not report growth rates (73 %), growth was determined from the plot of biomass over time in the article. However, if no plots or data were presented, the relationship $\mu = P B^{-1}$ was assumed valid.

Bioreactors were grouped into seven types: two open and five closed bioreactor systems. The two open systems were raceway ponds (RW, $n = 84$) and ponds ($n = 53$), and the five closed systems were vertical tubular reactors (VTR, $n = 30$), helical reactors ($n = 17$), horizontal tubular reactors (HTR, $n = 58$), flat plate reactors (FP, $n = 56$), and unique reactors (UBR, $n = 10$), which included cascades ($n = 5$), cones ($n = 3$), and dome/parabolic ($n = 2$). For bioreactors, both volume and surface areas were reported or calculated. Most studies (91 %) either reported the illuminated surface area of bioreactors or provided dimensions and orientation of bioreactors such that it was possible to calculate the surface area. For some studies of ponds and raceway systems where only the surface area was

given, the volume was calculated based on depth. If no depth was given, a mean depth of 0.1 m was assumed, based a nominal depth of the majority of these systems using data from Benemann [203] and Oswald [204]. For deeper ponds, of say 0.2 m, this would lead to twofold over-estimation of volume; however, this is still a small deviation in volume given the 2 to 10^6 L range of bioreactors studied.

While all articles reported species and/or genus, few gave the cell size or cell volume. Most, however, reported the origin of parent cultures, in which case cell size was found by searching the culture collection’s webpage for the target species’ dimensions. For articles where this was not possible, cell size was taken from Round [205] and for diatoms from Cupp [206]. Finally, cell volume was calculated based on cell morphology as a sphere or cylinder, with chains and colony size based on these two shapes.

Most studies cited the maximum biomass or provided graphs of biomass dry weight in grams per liter (g L^{-1}). Only 19 % reported cell number per culture volume (i.e., cell concentration). In this case, cell concentration was multiplied by the biomass per cell using Strathmann’s empirical relationship to convert to biomass [207]. Strathmann’s equation specifies pg dry weight carbon (C) per cell as a function of cell volume, and was converted to g dry weight biomass assuming that 52 % of the biomass was organic matter on a dry weight basis. Although the Strathmann regression applies to marine phytoplankton, the same trend occurs for freshwater species [208].

Results for bioreactor systems were compared with regressions of maximal growth rates, biomass, and production taken from the literature, which represent optimal conditions in laboratory cultures. Maximum growth rates were plotted against cell volume for diatoms [209], coccolithophores [210], green algae and cyanobacteria [211], and mixed species [212]. Biomass from bioreactors was compared to maximum cell concentrations from laboratory cultures [213] after converting concentration to biomass using Strathmann’s equation. Biomass was also compared to phytoplankton in temperate [214] and tropical [215] oceans after converting biomass per cell to g L^{-1} dry weight.

Regressions for biomass per cell (B_c), normalized biomass (B'), cell concentration (C), and maximum growth rate (μ_{max}) all followed a power law relationship, where

$$Y = aV_c^b \quad (1a)$$

Transforming to a log relationship, the equation for a line is as follows:

$$\log Y = \log a + b \log V_c, \quad (1b)$$

where Y is the parameter of interest, $\log a$ is the intercept, b the slope, and V_c is the cell volume in μm^3 . The units and regression variables a , b , and Y are given in Table 1.

Data from bioreactors were plotted for Eq. 1b, and confidence intervals were determined. To compare with regression data from bioreactors, confidence intervals for regressions in Table 1 were plotted for the original data points or were estimated from reported sample size and standard errors.

All statistical tests were done in Microsoft Excel using XLSTAT 2013 (V6.04) and StatPlus 2009 (V5.8) software. Turkey’s and Bartlett’s tests were used to compare variances between groups while a chi-squared test was used to determine normality of group distributions. Given equal variance and normality, differences between linear regressions were tested using analysis of covariance (ANCOVA). To compare the effect of different factors on cultures, analysis of variance was performed for equal variance distributions, otherwise the non-parametric Kruskal-Wallis test was employed or a Student t test if only two means were compared.

Results

Microalgae

For the 317 bioreactor cultures referenced in this paper, a total of only 35 genera were used. However, some algae have been studied more than others. Green algae have been used in almost half of the studies (45.6 %) with 19 % accounted for by

Chlorella spp. (Table 2). The second most studied class was cyanobacteria at 27 %, which was mostly *Spirulina* spp. (24 %). The unequal number of genera and species, some of which had small sample sizes, lead to unequal cell volume distributions for growth, biomass, and production regressions, as well as non-normality and unequal variances. Consequently, genera were grouped into classes to increase the sample size for statistical power.

Growth Rates

Maximal growth rates of microalgae have been found to be inversely proportional to cell volume (Table 1). Figure 1 shows that the highest growth rates in laboratory studies were for diatoms of all sizes followed by coccolithophores $>400 \mu\text{m}^3$. The lowest growth rates were for cyanobacteria and green algae, which were similar for both unicellular and colonies of all sizes.

Marañón et al. [212] found growth rates of mixed cultures (i.e., not class-dependent) were between diatom and coccolithophore maxima and only varied inversely with cell volume for the larger ($\geq 100 \mu\text{m}^3$) cell volumes. Smaller cells ($<100 \mu\text{m}^3$) displayed the reverse trend, although the magnitude of growth rates of these smaller cells varied between maxima for coccolithophores and green/cyanobacteria (Fig. 1).

For the different species grown in bioreactors, growth rates ranged from 0.01 to 4.8 day^{-1} . Although growth rates from bioreactors also had an inverse relationship to cell volume for

Table 1 Coefficients for regression of cell biomass, cell concentration, and maximal growth rates, all based on Eq. 1b

Parameter ($\log Y$)	Units	$\log a$	b	r^2	Reference
log Biomass					
B_c , Biomass cell^{-1a}	pg C DW cell^{-1}	-0.314	0.712	0.90	Strathmann [207]
B' , Biomass	mgC $\text{L}^{-1} \text{mg}^{-1} \text{cell}^{-1}$	-0.965	-0.158	0.96 ^b	Rodríguez and Mullin [214]
log C , cell concentration					
Laboratory ^b	cells mL^{-1}	8.79	-0.790	0.94	Agusti and Kalff [213]
Tropical ocean	cells mL^{-1}	3.61	-1.29	0.98	Huete-Ortega et al. [215]
log μ_{max} , Max. growth					
Green algae, total	day^{-1}	-0.164	-0.073	0.11	Adapted from Neilsen ^c [211]
Cyanobacteria, total	day^{-1}	-0.184	-0.067	0.11	Adapted from Neilsen ^c [211]
Diatoms	day^{-1}	0.580	-0.110	0.70	Banse [209]
Coccolithophores	day^{-1}	0.544	-0.32	0.86	Buitenhuis [210]
Mixed, $>100 \mu\text{m}^3$	day^{-1}	0.220	-0.150	0.86	Marañón et al. ^d [212]
Mixed, $<100 \mu\text{m}^3$	day^{-1}	-0.430	0.190	0.94	Marañón et al. ^e [212]

^a Independent of light or temperature regimes

^b Assumed light saturated cultures at $222 \mu\text{E m}^{-2} \text{s}^{-1}$

^c New regression on volume using original data based on diameter

^d Original regressions for $>40 \mu\text{m}^3$

^e Original regressions for $<300 \mu\text{m}^3$

Table 2 The percentage of dominant classes, genera, and cell types represented in this paper. Of the 35 genera in the 317 studies, genera in fewer than two studies were not listed here; hence, the total is 92.6 % and not 100 %

Class/genus	Percentage (%)	Cell type
Cyanobacteria	27	
<i>Spirulina</i>	24	Colonial
<i>Nodularia</i>	1	Colonial
<i>Synechocystis</i>	1	Unicellular
Diatoms	18.5	
<i>Phaeodactylum</i>	7.6	Unicellular
<i>Chaetoceros</i>	3.8	Chains
<i>Cyclotella</i>	1	Unicellular
<i>Fistulifera</i>	1	Unicellular
Green	45.6	
<i>Chlorella</i>	19	Unicellular
<i>Nannochloropsis</i>	9.7	Unicellular
<i>Tetraselmis</i>	5.5	Unicellular
<i>Scenedesmus</i>	4.8	Chains
<i>Haematococcus</i>	2.8	Unicellular
<i>Dunaliella</i>	2.4	Unicellular
<i>Micractinium</i>	1.7	Colonial
<i>Chlorococcum</i>	1.4	Unicellular
Prymnesiophyceae	6.2	
<i>Pleurochrysis</i>	3.1	Unicellular
<i>Isochrysis</i>	2.1	Unicellular
Other	2.1	
<i>Porphyridium</i>	1.7	Colonial
Total percent of classes	99.4	
Total percent of genera	92.6	

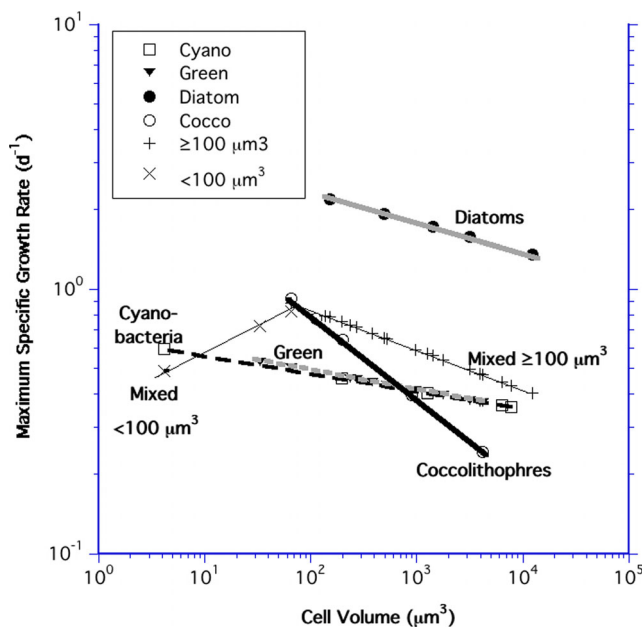


Fig. 1 Maximum growth rates as a function of cell volume based on Table 1

cyanobacteria, coccolithophores, diatoms, and green algae (Fig. 2), their low correlation coefficients indicated that cell volume did not explain the variation in growth (Table 3).

Confidence intervals for maximum and bioreactor growth rates of diatoms did not overlap (Fig. 2), indicating that growth in bioreactors was statistically lower than the maximum calculated by Banse [209]. The fact that confidence intervals for other algal classes did overlap, however, does not mean that maximum growth rates and those in bioreactors were not statistically different.

Even though growth rates were normally distributed for all the classes, the variance for coccolithophores was significantly lower than others ($X^2(3, 227) = 32.9$; $p < 0.0001$). Because of the unequal variance and the lack of linearity, growth curves could not be directly compared for the classes. However, cell volumes binned to similar sample sizes showed growth rates were not significantly different for algal classes (Table 4). Additionally, growth rates were not statistically different for binned cell volumes (Table 4).

Comparing means of growth rates in bioreactors was not as meaningful as assessing the highest growth rates achieved in the bioreactors. Indeed, it does not make sense to compare average growth rates in large bioreactor.

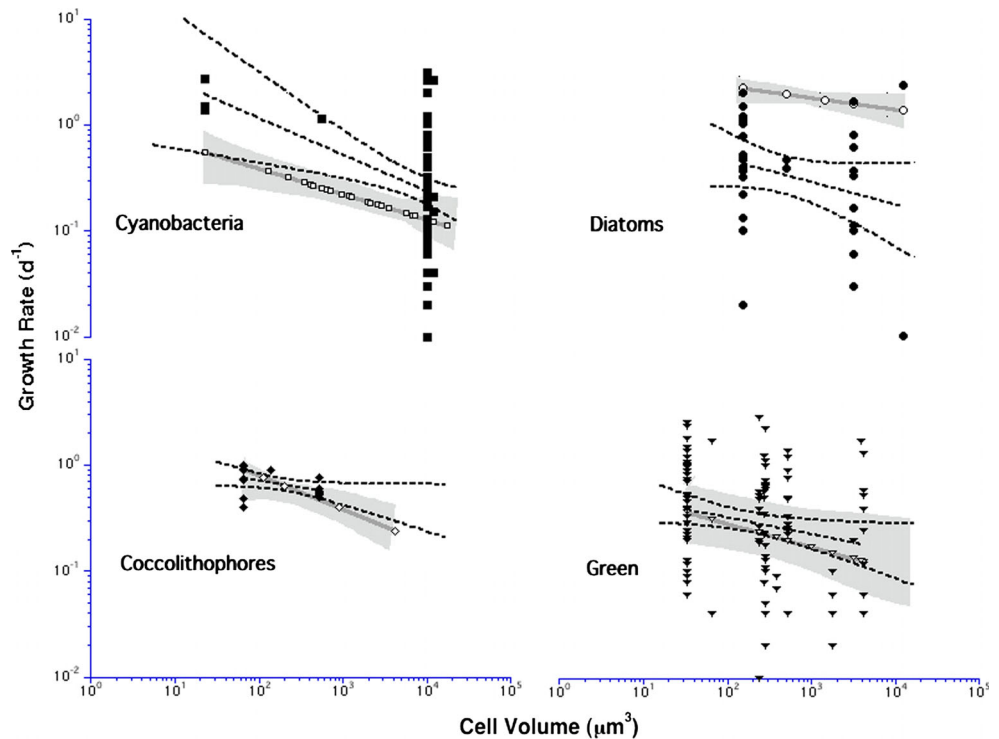
For small laboratory cultures, maximum growth rates for cell volumes $<500 \mu\text{m}^3$ were ranked as follows: diatoms $>$ coccolithophores $>$ cyanobacteria = green algae. In contrast, the highest growth rates in the bioreactors were ranked as follows: cyanobacteria = green algae = diatoms $>$ coccolithophores.

Overall, 71 % of the growth rates for the bioreactor studies were higher than maximum growth rates reported in the literature. Bioreactor growth rates exceeded maximal rates for 51 of 72 cyanobacteria, 14 of 18 coccolithophores, and 82 of 118 for green algae studies. Only once did the diatom growth rate in a bioreactor exceed the maximum rate, and this was in a bioreactor system under high light ($600\text{--}900 \mu\text{Em}^{-2} \text{s}^{-1}$) and low mixing for a short duration [113].

Biomass

The maximum, theoretical biomass (i.e., concentration) of an algal culture is given by the maximum number of cells packed per liter of culture and is based on the cell volume, and thus cell mass (Table 5). Several studies have shown that phytoplankton cell concentrations and standardized mass vary inversely with cell volume (and size), as seen by the negative slopes in Table 1. This is intuitive since a larger number of smaller cells can be packed into a liter compared to larger cells. However, Strathmann [207] showed that cell mass increases with cell volume (and size). Therefore, the maximum number of small cells in a liter of culture would have about the same biomass as fewer large cells in the same volume, and the maximum biomass should be constant for all cell sizes. Indeed, when converted to

Fig. 2 Growth rates from bioreactor cultures (solid symbols, dashed regression lines) and maximum growth rates (open symbols, gray regression lines) versus cell volume. Confidence intervals are plotted at the 95 % level for maximum growth rates (shaded in gray) and bioreactor systems (dotted lines). Regression constants for cultured systems are presented in Table 3



biomass in g L^{-1} using Strathmann’s equation, this inverse relationship of cell concentration on cell volume is much weaker (Table 4) since the mass per cell increases with cell volume making the slopes of biomass per cell volume less negative (Fig. 3).

The regression of bioreactor biomass over cell volume had a low correlation coefficient, and the slope was not statistically significant (Tables 5 and 6). Results of the ANCOVA for biomass comparisons showed a slight statistical significance ($P > 0.033$), which was attributed to growth rate ($|t| > 0.015$) but not to class or binned cell volume (Table 7).

Despite the large degree of scatter in bioreactor biomass, values were less than the packed cell biomass, but much greater than ocean populations, and overlapped the maximum biomass for laboratory data. Still, bioreactor biomass exceeded the maximum laboratory biomass in 86 % of the samples. The maximum biomass and the biomass in the bioreactors were orders of magnitude greater than distributions in the natural environment, most likely because in aquatic environment, loss rates are higher (e.g., herbivory and bacterial/viral infects), and optimum growth conditions are seasonal.

Production Rates

The linear dependence of production rate on biomass and growth rate was compared, as was algal class and binned cell volume. Productivity was not statistically significant for binned cell volume or for algal class but did vary with both biomass and growth rate (Table 8). The fact that biomass is related to growth rate means that production could be directly affected by growth rate and indirectly affected by the influence of growth on the maximum biomass. However, it could also indicate that production is directly affected by biomass and that biomass affects growth, say by shading cells and reducing optimum growth conditions.

The maximum production rate in full sunlight has been estimated at $173 \text{ g C m}^{-2} \text{ day}^{-1}$ for cultures grown on nitrate as a nitrogen source and about $200 \text{ g C m}^{-2} \text{ day}^{-1}$ for ammonium [216, 217]. The later gives a biomass production of approximately $400 \text{ g m}^{-2} \text{ day}^{-1}$ or $4 \text{ g L}^{-1} \text{ day}^{-1}$ for a bioreactor with a 10:1 surface to volume ratio ($400 \text{ g m}^{-2} \text{ day}^{-1} \times 10\text{m}^2 \text{ m}^{-3}/1000 \text{ L m}^{-3}$), which is within the range calculated by Williams and Lauren [199].

Table 3 Regressions of bioreactor growth rates as a function of cell volume, V_c , for the four major algal classes

	<i>F</i>	<i>P</i>	SS	<i>n</i>	log <i>a</i>	<i>a</i>	<i>b</i>	<i>r</i> ²
Cyanobacteria	10.6	0.002	2.47	72	0.466	2.922	−0.282	0.131
Coccolithophores	4.21	0.058	0.05	17	0.127	1.340	−0.124	0.167
Diatoms	0.44	0.512	0.10	35	−0.214	0.611	−0.084	0.013
Green algae	6.93	<0.01	1.89	116	−0.040	0.912	−0.195	0.049

Table 4 ANCOVA results for growth rates by class and binned cell volumes

Source	df	SS	MS	F	P
Class	4	8.176	2.044	1.363	0.247
Error	237	355.36	1.499		
Total	241	363.54			

Production in bioreactors varied from 2×10^{-3} to $12 \text{ g L}^{-1} \text{ day}^{-1}$ with biomass changes from 3×10^{-3} to 67.3 g L^{-1} and growth rates from 10^{-2} to 4.8 day^{-1} (Fig. 4a). Mean values were $0.74 \text{ g L}^{-1} \text{ day}^{-1}$, 3.4 g L^{-1} , and 0.61 day^{-1} for production, biomass, and growth, respectively. Although it may seem odd to plot biomass and growth as a function of production (and not vice versa), this clearly shows that biomass was more correlated than growth rate to production as seen by the slope of the regression lines in Fig. 4a.

Additionally, for production rates $\geq 1 \text{ g L}^{-1} \text{ day}^{-1}$, the mean growth rate was 0.79 day^{-1} , which was only 18 % greater than the overall mean, while the mean biomass was 8.7, nearly triple that of the overall mean biomass. All these trends indicate that production was dominated by biomass, and not growth rates, which were much more constrained. Further, it appears that for high biomass, growth rates declined, probably as light or nutrients or both become limiting (Fig. 4b).

Bioreactor Types

Production rates for open ponds and raceway bioreactors had lower variances than closed bioreactors and significantly lower mean production ($\chi^2 = 70.7$, $P < 0.0001$) compared to the higher production rates in closed bioreactors (Fig. 5a), which was also found by Williams and Lauren [199]. However, variances for production in the five types of closed bioreactors were not different, and neither were their mean production rates (Fig. 5b), ($df = 156$, $F = 0.849$, $P = 0.496$).

Unfortunately, few algal species have been grown in all types of closed bioreactors making comparisons difficult.

For species that have been cultured in more than one bioreactor type, statistical analysis was hampered by low samples size for one or more bioreactor types. Therefore, in some cases, species were grouped into classes to increase sample size to make comparisons possible. Figure 6 shows that production across algal classes was not statistically different in either open ($df = 194$, $F = 0.7105$, $P = 0.5468$) or closed systems ($df = 191$, $F = 0.7105$, $P = 0.547$). As in Fig. 5a, closed bioreactors had significantly higher production than open systems, independent of algal class ($df = 297$, $F = 3.529$, $P = 0.001$).

Comparing production for bioreactor type (Fig. 7), the only statistical difference was in the horizontal tubular reactors (HTR), where *Chlorella* production was significantly higher than cyanobacteria production ($df = 42$, $F = 7.182$, $P = 0.001$). Species means for flat plates ($df = 42$, $F = 0.569$, $P = 0.638$), VTR ($df = 23$, $F = 1.24$, $P = 0.309$), and helical tubes ($df = 6$, $F = 1.087$, $P = 0.985$) were not significantly different. When comparing production within a species cultured in different bioreactors, only *Chlorella* had a mean P significantly larger for HTR than for FP and VTR ($df = 33$, $F = 5.641$, $P = 0.008$). No differences in P were determined for diatoms ($df = 25$, $F = 0.900$, $P = 0.4567$), *Nannochloropsis* ($df = 17$, $t = 1.663$, $P = 0.114$), cyanobacteria ($df = 13$, $t = 1.666$, $P = 0.119$), or *Tetraselmis* ($df = 16$, $t = 2.1767$, $P = 0.0443$) cultured in different bioreactors. Generally, it seems that most microalgae grow equally well in most bioreactors.

Looking at the effects of physical characteristics of bioreactors, production rate was inversely related to the volume of the bioreactor (Fig. 8a) and directly proportional to surface area (not shown). The relationships for production were statistically significant at the 95 % level ($df = 256$, $F = 16.585$, $P < 0.0001$) for both volume and surface area of the bioreactors (Table 9). Interestingly, production varied directly with surface to volume ratio (Fig. 8b), though the trend had a lower correlation coefficient ($r^2 = 0.0484$, $df = 265$, $F = 4.73$, $P = 0.030$). There was a significant difference in production

Table 5 Regressions of biomass ($\text{g L}^{-1} \text{ dwt}$) for bioreactor cultures and lab and ocean populations

	F	P	SS	n	log a	a	b	r ²
Culture systems	0.59	0.44	0.166	249	-0.942	0.114	-0.018	0.006
Packed cells ^a					2.987	970.6	-0.288	
Lab. cultures ^b					-0.524	0.299	-0.078	
N. Pacific Gyre ^c					-6.143	7.2×10^{-7}	-0.112	
Tropical Atlantic ^d					-5.824	1.5×10^{-6}	-0.348	

^a Based on Table 6

^b Cells L^{-1} from Agustí and Kalfi [213] converted to biomass, in g L^{-1} , using g cell^{-1} from Strathmann [207]

^c Biomass from Rodríguez and Mullin [214] converted to g L^{-1} from Strathmann [207]

^d Cells L^{-1} from Huete-Ortega et al. [215] converted to biomass, in g L^{-1} , using g cell^{-1} from Strathmann [207]

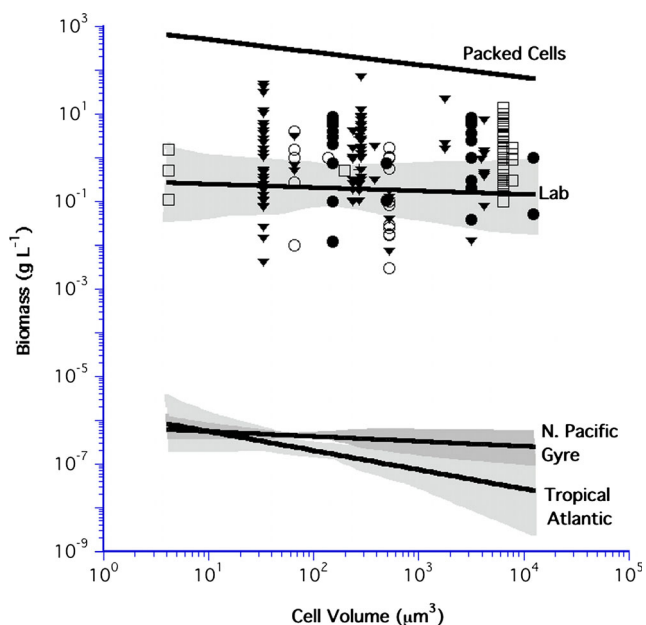


Fig. 3 Biomass for culture systems for cyanobacteria (□), coccolithophores (○), diatoms (●), green algae (▼), and other (+) relative to limits of biomass for packed cells and populations in oceans and the laboratory. Confidence intervals are plotted at the 95 % level

for open systems and three of the closed bioreactors (VTR, HTR, and FP) with the flat plates having the highest production rates (Table 10).

Bioreactor volume and illuminated surface area (SA) are physical variables that depend on the design of the culture system. Both directly affect production, and thus oil yield. For instance, a production rate of 1 g L⁻¹ day⁻¹ in a 10²-L bioreactor would yield a biomass of 10² g day⁻¹, while the same production rate in a 10⁶-L pond would yield 10⁶ g day⁻¹. Surface area of the culture system is important since it determines the photon flux to the cultures, while the ratio of surface to volume relates to the light path.

Figure 9a shows that surface area was highly dependent on the volume of the bioreactor (df = 265, F = 1360, P < 0.0001) with most of the variance accounted for by the volume (r² = 0.880). However, this increase in surface area was accompanied by a reduction in the surface to volume ratio,

which decreased from about 10²:1 to <1:1 m² m⁻³. This change in the surface to volume ratio is more clearly shown in Fig. 9b

Predicted Oil Yield

The oil yield from algal cells depends not only on production rate and culture volume but also on the mass of lipid droplets in the cells (species-specific) and the efficiency of extracting them. Yield is given by:

$$\text{Yield} = PV S_o e_o \rho_{oil}^{-1} \tag{2}$$

where Yield is oil in L day⁻¹, P is production (g L⁻¹ day⁻¹), V is culture volume (L), S_o is oil storage as percent biomass, e_o is extraction efficiency, and ρ_{oil} is the oil density. By keeping species-specific oil storage constant and extraction efficiency fixed, it is possible to compare oil yields based only on bioreactor characteristics. Oil yields were calculated based on P and V for bioreactors under the assumptions that 30 % of the biomass was oil [192], the wet extraction efficiency was 70 % [218], given an algal wet weight to dry weight of 12-fold [219], and ρ_{oil} was 900 g L⁻¹. Of course, more efficient downstream processing and use of algal strains with higher oil content or optimal fatty acid profiles would give higher oil yields; however, since this review is focused on bioreactor processes, these topics are beyond the scope of this paper. Further, a statistical analysis of fatty acids across species and bioreactor type would be handicapped by few data points, since this type of data has only recently been published as a result of new and improved analytical techniques.

Oil yield was highly correlated to culture volume, such that larger volumes yielded more oil (Fig. 10a), which was opposite to the trend in production, which decreased with increasing bioreactor volume (Fig. 8a). However, oil yield decreased with increasing surface to volume ratio (Fig. 10b).

Productivity and yield were categorized into four groups of bioreactors: (1) higher yield and lower production (28 %); (2) higher yield and higher production (11 %); (3) lower yield and

Table 6 Characteristics of cell sizes in a 1-L culture volume

Cell diameter (μm)	Cell volume ^a (μm ³ cell ⁻¹)	Packed concentration ^b (cells L ⁻¹)	Packed cell biomass ^c (g L ⁻¹)
1	0.52	1.9 × 10 ¹⁵	5.8 × 10 ²
10	5.2 × 10 ¹	1.9 × 10 ¹³	1.6 × 10 ²
10 ²	5.2 × 10 ³	1.9 × 10 ⁹	4.1 × 10 ¹
10 ³	5.2 × 10 ⁵	1.9 × 10 ³	1.1 × 10 ¹

^a For spherical cells, V_{cell} = πd³/6, where d is the diameter in μm

^b For C packed, calculated as 10¹⁵ μm³ L⁻¹/V_c

^c Biomass calculated from Strathmann [207] as B_c (g cell⁻¹) × C (cells L⁻¹)

Table 7 ANCOVA results for biomass (*B*) by class, growth rate, and binned cell volume

Source	df	SS	MS	<i>F</i>	<i>P</i>
Model	5	759.12	151.825	2.475	0.033
Error	236	14,475.20	61.336		
Total	241	15,234.30			
				<i>t</i>	<i>P</i>
Cell volume	241	0.000		−1.217	0.225
Growth rate	241	0.415		−2.459	0.015
Cyano <i>B</i>	241	1.752		−0.568	0.571
Cocco <i>B</i>	241	1.999		−1.179	0.240
Diatom <i>B</i>	241	1.545		−0.976	0.330
Green <i>B</i>	241	0.000			

higher production (37 %); and (4) lower yield and lower production (24 %). Figure 11a shows the grouping based on these four criteria, where high yield is assumed to be ≥ 1 L day^{−1} and high production to be one-tenth of the theoretical maximum (i.e., ≥ 0.4 g L^{−1} day^{−1}), which was true for all bioreactors except for six ponds that had higher production but were clearly not clustered with group 2, and hence were assigned to group 1.

Only 36 % of the bioreactor systems achieved yields >1 L day^{−1}; thus, most of the bioreactors were low yield. Bioreactors with large aerial footprints (i.e., ponds, raceways, and horizontal tubular systems) dominated group 1 (89 %). Group 2 was composed of closed bioreactors or intermediate size, except for one small (2200 L) raceway pond. Group 3 was mostly small, closed bioreactor systems, and group 4 had a mix of bioreactor types and sizes.

The group median (mean) volumes were as follows: group 1, 10⁵ L (8×10^7 L); group 2, 395 L (665 L); group 3, 33 L (85 L); and group 4, 200 L (3780 L). Excluding raceway ponds, the closed bioreactors in the group 2 had volumes

Table 8 ANCOVA results for production (*P*) by class, biomass, growth, and binned cell volume

Source	df	SS	MS	<i>F</i>	<i>P</i>
Model	6	236.77	39.46	16.25	<0.0001
Error	234	568.28	2.429		
Total	240	805.05			
				<i>t</i>	<i>P</i>
Cell volume		0.000		−0.604	0.546
Biomass		0.013		5.558	<0.0001
Growth rate		0.083		7.041	<0.0001
Cyano <i>P</i>		0.352		2.652	0.009
Cocco <i>P</i>		0.398		0.743	0.458
Diatom <i>P</i>		0.315		1.616	0.107
Green <i>P</i>		0.000			

ranging from 140 to 1400 L and surface to volume ratios of up to 10²:1, as indicated by the shaded region in Fig. 11b.

Of the 19 closed bioreactors in the group 2, about 50 % had surface to volume ratios of approximately 10² or higher. Of these ten bioreactors with high SA:V, four were horizontal tubular reactors (HTR), four were helical, one was a vertical tubular (VTR), and one was an inclined cascade. The horizontal tubular system and inclined cascade were bioreactor systems that require large horizontal surfaces to optimize the bioreactor-illuminated surface area relative to the nadir angle of the sun, and therefore, they had large aerial footprints. Helical systems had high illuminated surface areas but also had a large footprint resulting from the extensive curvature of the tubes around an inner column, which was not part of the bioreactor volume, meaning it is an unproductive part of the footprint. The smallest aerial footprint was the vertical tubular bioreactor since its cylindrical column, which is the productive core of the bioreactor, was tall but had a small diameter. Several other reactors exhibited high surface to volume ratios (i.e., flat plates); however, these had smaller volumes or lower production rates (Fig. 11b).

Conclusions

Algae Diversity

Over the nearly 200 years that algae have been studied, their taxonomy is still being altered, which makes tallying the number of species difficult [205]. Despite the difficulties in assessing algal characteristics, the Algal Database lists over 135,000 species [220] of which seaweeds are included, and some species may have duplicate taxonomic names. In all, there are 15 distinct phyla of algae.

Despite the large number and diversity of microalgal species, few have been grown in larger (≥ 3 L) bioreactors. Thus, the 35 genera represented here are only a small fraction of the known microalgal species, and even these species have different genomes with varied traits. Early work by the Aquatics Species Program studied nearly 3000 algal species but only 300 were cultivated [61]. In 2008, the Food and Agricultural Organization estimated that 40 species were used commercially [221] worldwide, although Terdici et al. [222] reported 10 species were commercial harvested in large bioreactors.

There are many types of photosynthetic organisms that could be used to produce vegetable-like oils including macrophytes, aquatic vascular plants, phytobenthos, as well as phytoplankton, which inhabit freshwater, marine, and terrestrial environments. One of the difficulties in finding data on these oil-producing plants and algae is that studies are distributed between different disciplines, the predominant ones being: oceanography which is concerned with marine species; limnology which focuses on freshwater species; phycology which looks at physiology and

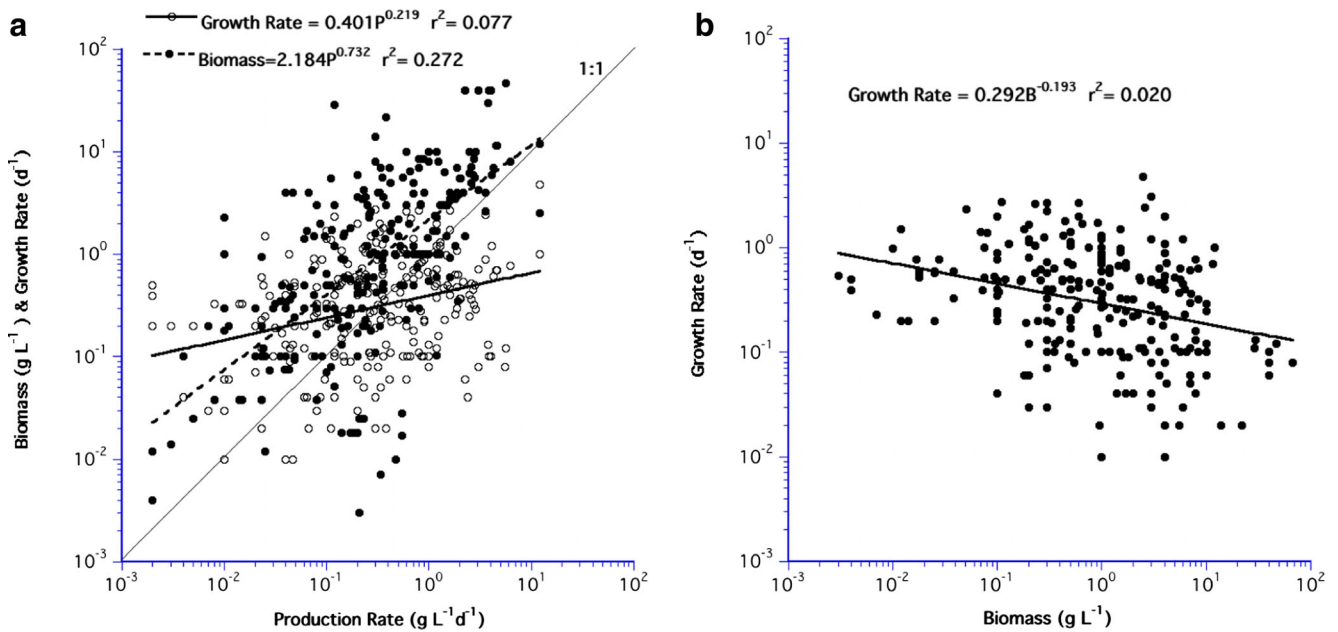


Fig. 4 The relationships between **a** production rate based on biomass (●), and growth rate (○) and **b** growth rate versus biomass. The *dashed line* is the slope for biomass; the *heavy, solid line* for growth rate. The *light, solid line* represents the 1:1 relationship

morphology; and applied phycology and biotechnology which study the industrial and practical aspects of algal science and bioengineering. Therefore, the search for the best oil-producing species/subspecies is disparate and far from complete. Yet, even if cell lipid storage could be doubled by more lipid-rich species or through genetic [223] or physiological [224] manipulation of cells, the overall effect on oil yield would not be as great as

optimizing biomass concentrations and production rates, which could increase yields by tenfold or more.

Growth Rate, Biomass, and Production

Prior laboratory experiments found that maximum growth rates of microalgal cells were predominately dependent on

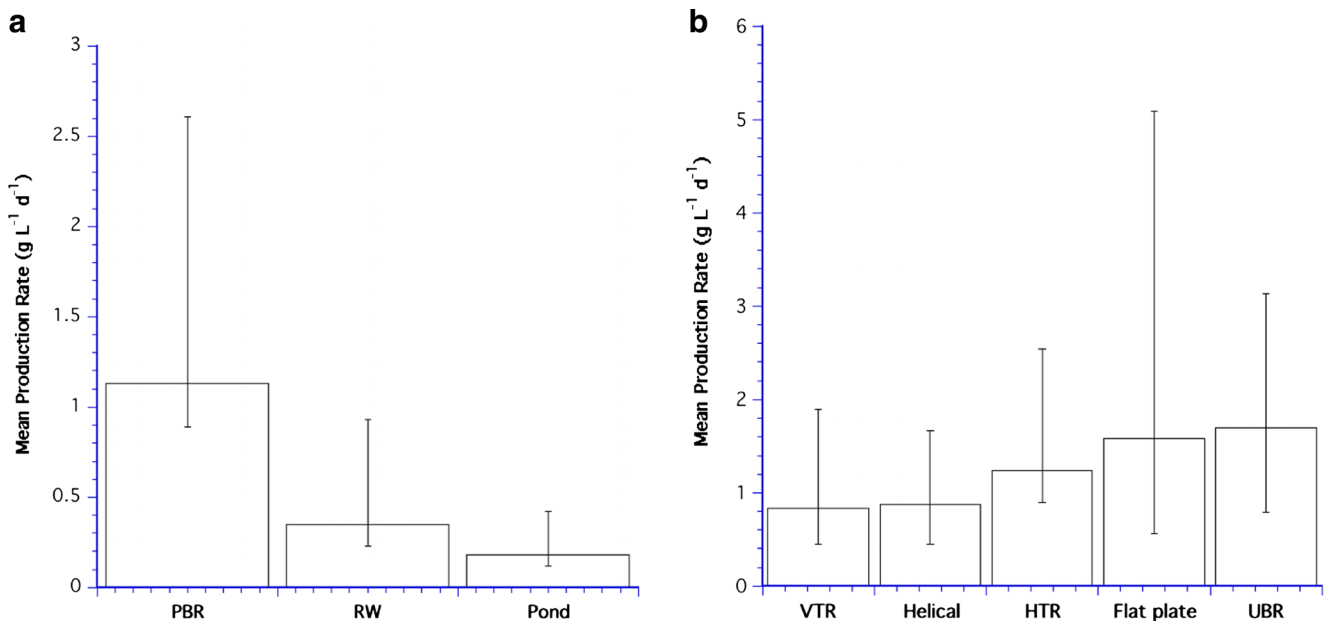


Fig. 5 Mean production rates for **a** photo-bioreactors (PBR), raceways (RW), and ponds; and **b** the five different types of photo-bioreactors: vertical tubular reactors (VTR), unique reactors (UBR), helical reactors,

horizontal tubular reactors (HTR), and flat plate reactors (FP). The *upper bar* is the standard deviation, and the *lower bar* is two standard errors

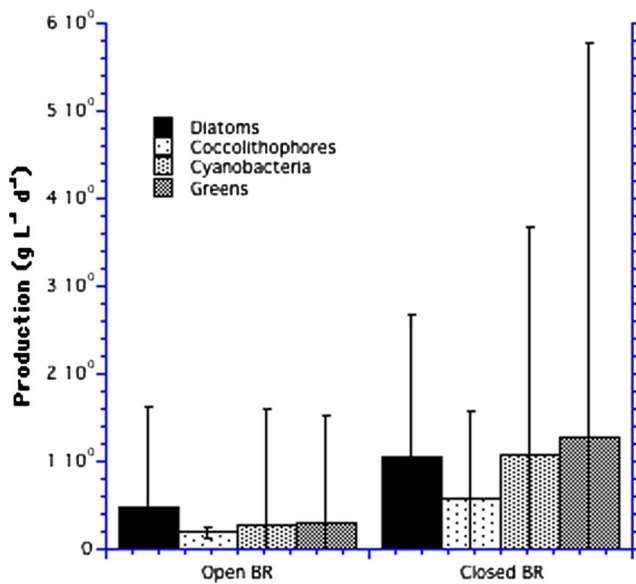


Fig. 6 Production rates for diatoms, coccolithophores, cyanobacteria, and green algae in both open and closed bioreactors (BR). Error bars are two standard deviations

the species and their cell size. In contrast, growth rates in bioreactors showed less species difference and no significant tendency to decrease with increasing cell size. The fact that growth in bioreactors varied so dramatically for the same species probably obscured any trend in growth rate with cell size. All the determinations of maximum growth in laboratory settings used only a few species, in small volumes, and with few variations in culture conditions (media, light, CO₂, mixing,

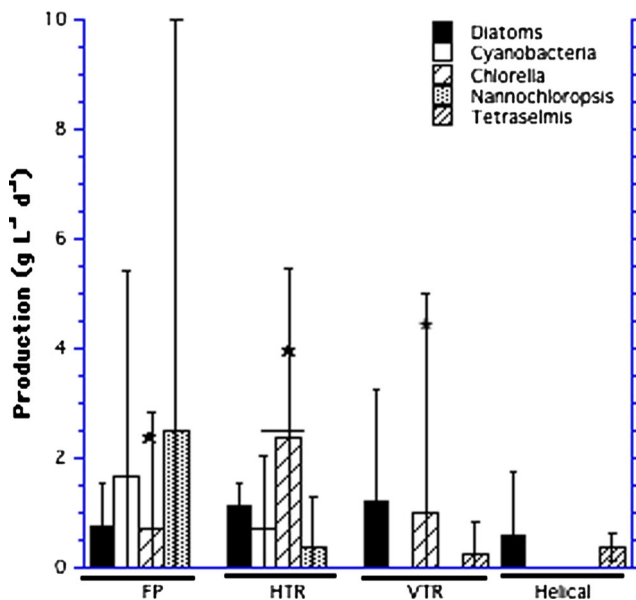


Fig. 7 Production for three green algae (*Chlorella*, *Nannochloropsis*, and *Tetraselmis*) cultured in flat plate (FP), horizontal tubular reactor (HTR), vertical tubular reactor (VTR), and helical reactor. Error bars are two standard deviations. Stars indicate a significant difference for *Chlorella*, and the bar in HTR represents a significant difference between algae cultured in the HTR

etc.). In contrast, growth rates in the bioreactors were determined for many more species, over a wider variety of growth conditions. In general, the fact that growth rates in bioreactors exceeded maximal growth rates, especially for green algae and cyanobacteria, may indicate that optimum conditions occurred in these bioreactors but not for the laboratory cultures used to determine the maximum growth rates. Still, growth rates for diatoms in bioreactors were generally lower than the maximum rates, which may indicate that some bioreactors had sub-optimal growth conditions for the species cultured. It is clear that bioreactor-specific growth rates never exceed 5 day⁻¹, so growth rate was very confined in relation to production.

Biomass in the bioreactors was nearly constant over cell size. For all but 14 % of the samples, bioreactor biomass was greater than the maximum biomass derived from laboratory data, although less than the packed concentration. The mean biomass was 3.5 g L⁻¹ with a median value of 1.0 g L⁻¹, and the fact that the slope of biomass versus cell size was flat means that mass was constant such that the same mass could be achieved using a large number of small cells or fewer large cells. Therefore, the selection of a target species should not necessarily depend on cell size but on growth rate, oil content, and the specific culture conditions needed to maximize the biomass.

Because population growth requires the synthesis of new biomass, μ_{\max} is closely related to metabolic rate [225, 226] with the same unimodal size scaling in CO₂ fixation, which represents a biomass turnover rate. A unimodal size scaling of biomass-specific metabolic rate corresponds to a curvature in the log–log relationship between individual metabolic rate and body size [227, 228]. Thus, the decrease in maximum growth rates with increasing cell volume can be explained by resource limitation as a function of surface to volume ratio [212]. Because the half-saturation coefficient, k_s , for nitrate uptake increases with mean spherical cell diameter, large cells with lower surface to volume ratios have higher k_s , and slower nitrate uptake rates.

Chisholm [229] speculated that the maximum biomass and maximum growth rate could be related to cell nutrient requirements of microalgae. Using the diffusion limitation model of Morel et al. [230], she determined the nitrogen requirement for a spherical cell. The steady state, diffusion flux of nitrogen, with a nitrate concentration C to a cell of diameter, d , is given by $J = 2\pi dDC$, where D is the molecular diffusion coefficient of the nutrient (10⁻⁵ cm² s⁻¹). Chisholm noted that $J \geq \mu Q_N$ was necessary for a cell to grow, where Q_N is the cell quota of nitrogen in moles N cell⁻¹. Q_N was determined from carbon content of the cell using the Redfield ratio of 16C:N for cells in exponential growth and the Strathmann's equation to relate carbon content to cell volume, $\pi d^3/6$. Recasting J in terms of

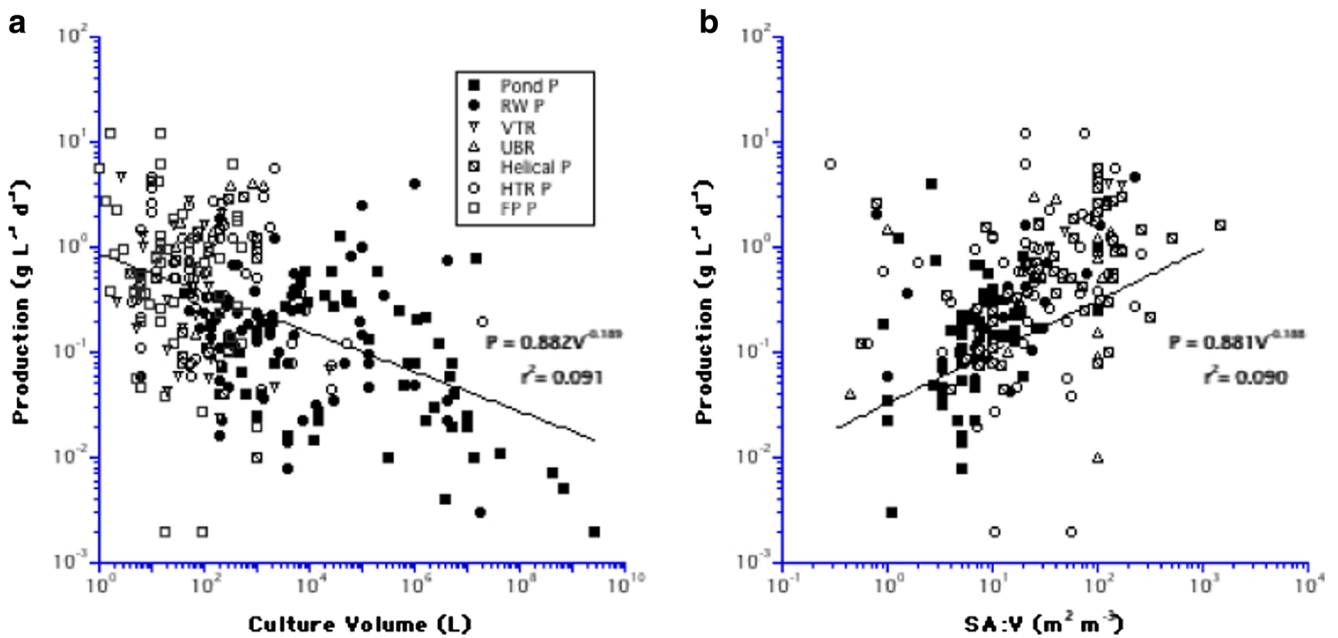


Fig. 8 **a** Production versus the volume of the culture system. The seven types of bioreactors systems are ponds (■), raceway ponds (RW, ●), vertical tubular reactors (VTR, ▽), unique reactors (UBR, △), helical

reactors (⊠), horizontal tubular reactors (HTR, ○), and flat plate reactors (FP, □); and **b** production versus the surface to volume ratio

μ , gives $\mu = 2\pi dDC Q_N^{-1}$ or based on cell volume, $\mu = 2\pi dDC(1.3 \times 10^3 d^{2.16})^{-1}$, which is proportional to $\mu \propto DCd^{-1.16}$. This equation indicates that for balanced growth, the nutrient concentration must increase as cell size increases. Thus, to maintain a growth rate of 1 day^{-1} , smaller cells require lower concentrations of nutrients than larger cells. The maximum biomass for laboratory cultures was reached when cells were spaced roughly $10 \text{ }\mu\text{m}$ apart [229]. A nutrient concentration of $10^{-1} \text{ }\mu\text{M N-NO}_3^- = 10^{-7} \text{ mol N L}^{-1}$ has 6×10^{16} molecules in a liter (6.02×10^{23} molecules $\text{mole}^{-1} \times 10^{-7} \text{ mol N L}^{-1}$) or 60 molecules in $1 \text{ }\mu\text{m}^3$. This is approximately one molecule every $0.25 \text{ }\mu\text{m}$. However, as the nutrient concentration increases so will the number of molecules per unit volume, thus the distance between molecules will decrease, and more molecules will be closer to the algal cell. Biomass in the bioreactors was greater than the maximum laboratory biomass and presumably was not limited by nutrients. For the high biomass concentrations in bioreactors, the

distance between cells would decrease, and cells would be closer to nutrient molecules. This is important since the distance over which diffusion acts would be shorter, so that nutrient limitation could be avoided and growth and biomass stimulated, providing the nutrient concentration was sufficient to meet the cell’s growth quota.

Diffusion-limiting conditions can also be overcome by enhancing the differential motion between the fluid and the cell [231, 232]. This differential motion reduces the boundary layer around the cell and effectively increases the diffusion flux to the cell. The most common means of creating motions in cultures is by cell sinking [233, 234] and by mechanical mixing [235, 236].

Mixing rates and biomass also play a role in light exposure of the cells. Production has been shown to vary with high-light conditions. Increased mixing of increasingly concentrated cultures also enhances production [158, 163, 236], presumably by increasing the exposure time of the cells to the light regime. Previous to this, Laws et al. [48] increased production in a raceway pond using turbulent eddy shedding to create high-frequency fluid motions, thereby exposing cells to high-light fluctuations.

However, as biomass increases, so does attenuation of light in the bioreactor. Light attenuation can be modeled as $k = a + b$, where a is absorption, mostly due to algal pigments and water, and b is scattering due to cell biomass and water. Mie scattering theory predicts that smaller particles scatter light at shorter wavelengths. Whitmire et al. [237] found that b depended on both cells size and cell shape. They also determined that chlorophyll concentrations were highly correlated

Table 9 ANCOVA for production rate on bioreactor volume and surface area

Source	df	SS	MS	F	P
Model	9	50.37	5.597	16.58	<0.0001
Error	256	86.39	0.3371		
Total	265	136.76			
				<i>t</i>	<i>P</i> > <i>t</i>
Culture volume		0.054		-6.612	<0.0001
Surface area		0.059		4.712	<0.0001

Table 10 ANOVA for production rate (P) for bioreactor surface to volume ratio

Source	df	SS	MS	F	P
Total	556	2,223,485	3999	10.9611	<0.0001
Type	7	272,647	38,949		
Error	549	1,950,837	3553		
Tukey's test					
SA:V comparison	Mean difference	q	P		
Pond P	44.84	7.07	<0.0001		
RW P	44.68	8.31	<0.0001		
VTR P	44.19	5.44	0.0033		
FP P	43.88	6.58	0.0001		
HTR P	43.78	7.06	<0.001		

to b for different species. Scattering by cells, however, is more concentrated in the forward direction [238] and directly proportional to cell size [239]. High pigment concentrations tend to increase a in cultures which also increases with cell size [240]. Hu et al. [166] showed that the higher the cell concentration in a culture, the higher the attenuation of light, with no more than a 1 mm of light (at 550 nm) penetration for a biomass of 15 g L⁻¹. The optimum light path length to maintain high production for small cells was 20 cm [183].

Inconsistencies in biomass yields for microalgal systems are largely responsible for the economic uncertainty and unfavorable results of life cycle analyses for biofuel production [241]. However, microalgae-based biofuels also hold the future possibility for providing energy independence from fossil

fuels, without compromising the use of arable lands or food production [200, 242, 243]. Benemann and Oswald [4] and Linquist et al. [243] argue that a biomass of 20 g m² day⁻¹ with an oil content of 25 % oil costs more than the market value, and needs to be ≥ 30 g m² day⁻¹ with a content of 50 % oil. For a SA:V of 10²:1 this would be 3 g L⁻¹ day⁻¹, which is similar to a recent estimate by Griffiths et al. [244] for cells with 50 % oil content, but much lower for cells with lower oil content.

Bioreactors and Scale-up

Production in algal cultures decreases with the volume of the bioreactor, and open bioreactor systems are not as productive

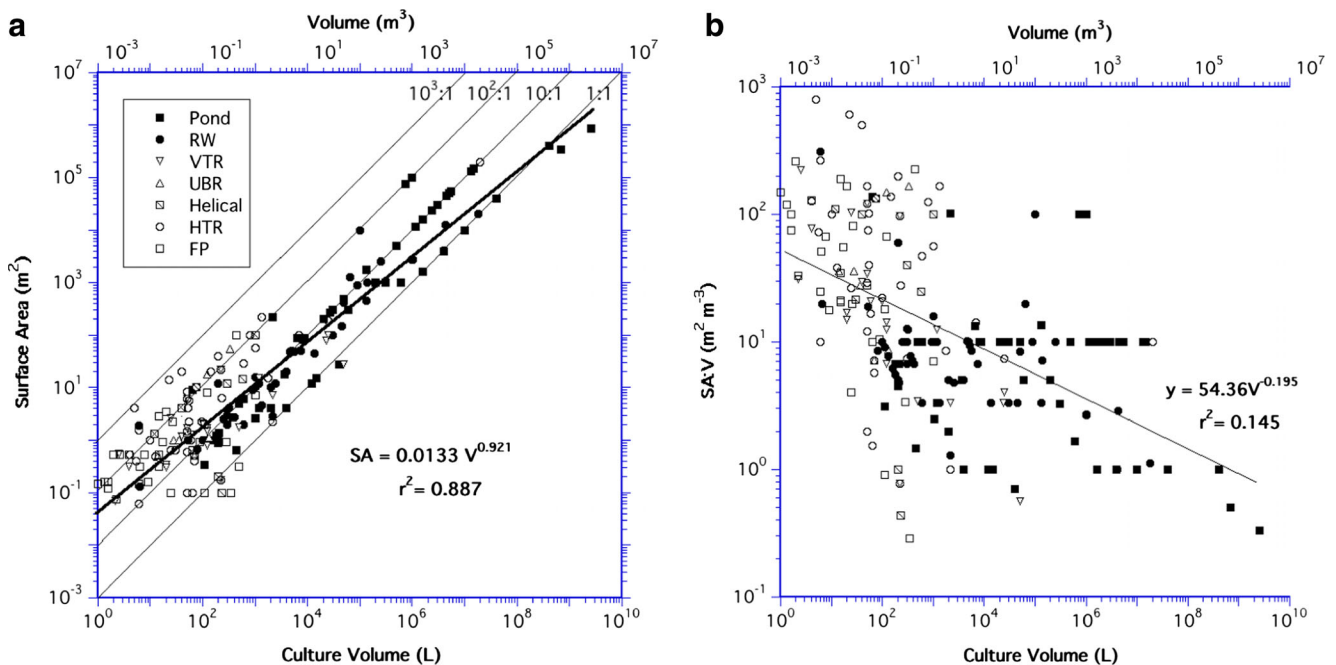


Fig. 9 Regressions for surface area on bioreactor volume (a) and surface to volume ratio of bioreactors (b). The *thick line* in a is the regression fit while the *thin lines* indicate the surface area to volume ratios from 1 to 10³ m² m⁻³

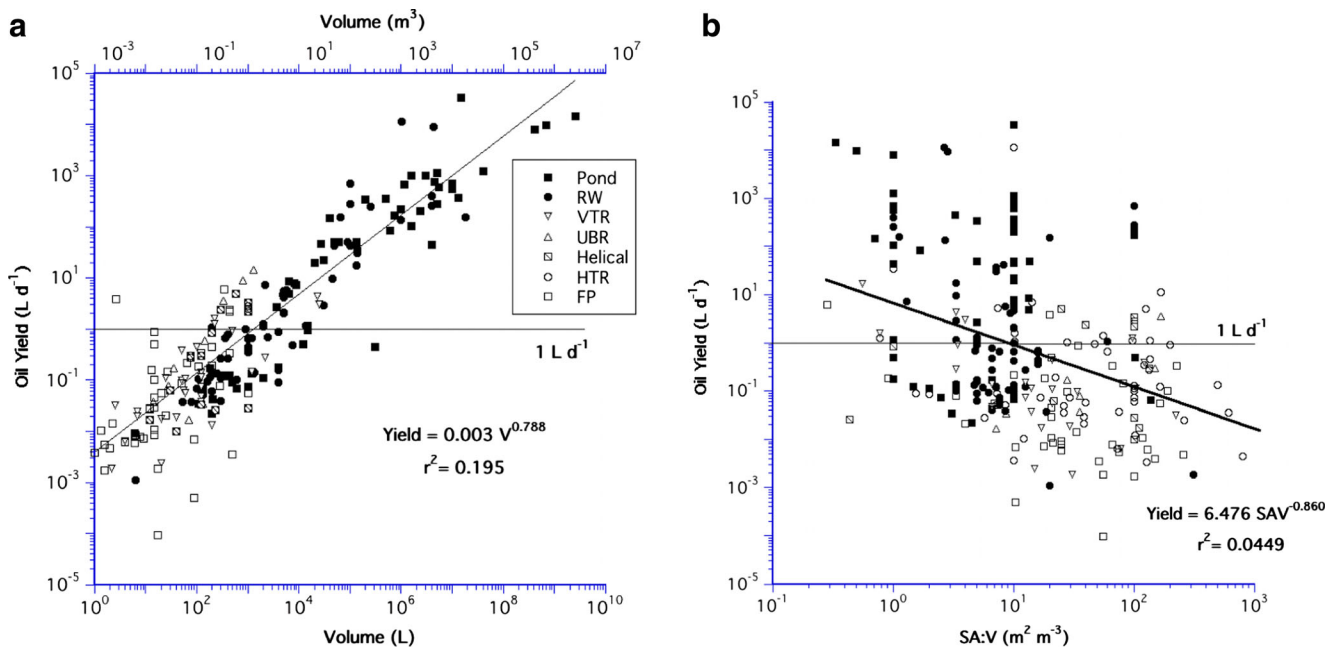


Fig. 10 Oil yields based on bioreactor: **a** volume; and **b** surface to volume ratio

as closed ones. However, production in different types of bioreactors may be closely associated with bioreactor volume since the median volume for open systems was 4000 L ($N = 136$) compared to a median volume of 55 L ($N = 182$) for closed systems. There also seems to be no strong data to support that one species grows best in a specific bioreactor type. Many scientists assume that species production is

dependent on bioreactor type, but this does not seem to be supported by these statistical analyses. It is more likely related to bioreactor operations.

Benemann [245] estimated 90 % of microalgae production worldwide is in ponds, most of which are in China. Large ponds that produce algal biomass for biofuels often exceed 10^5 L. However, such systems have come under increasing

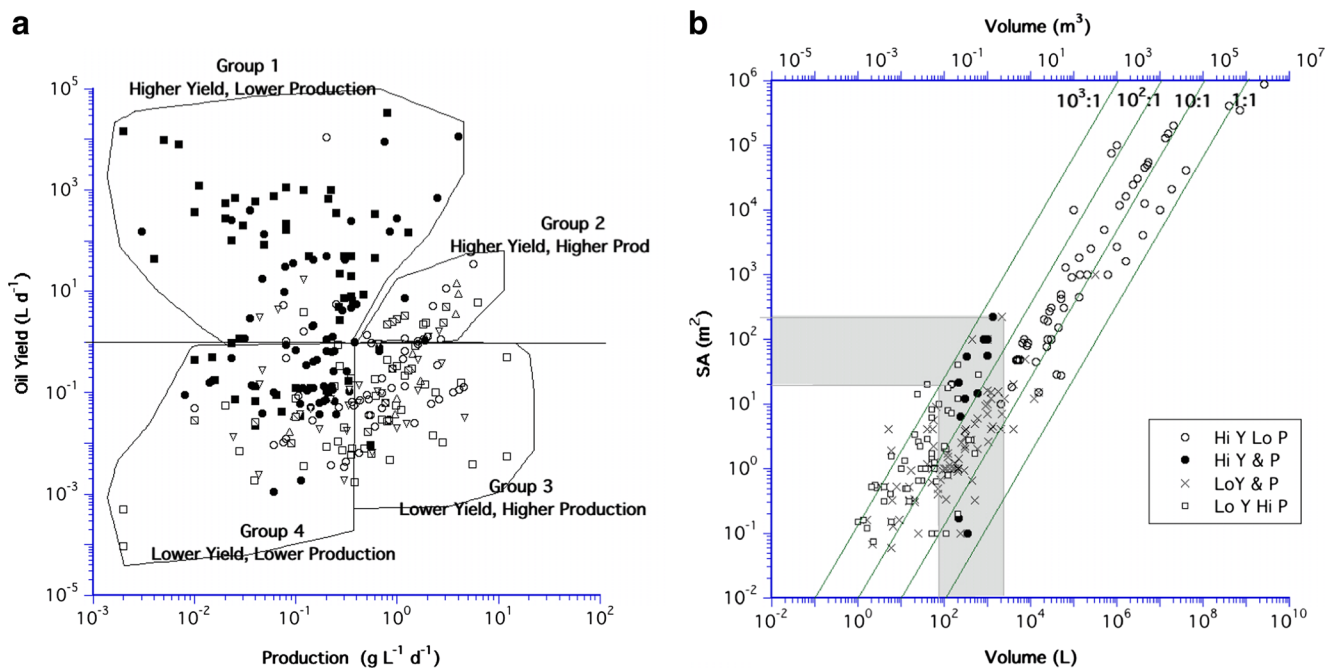


Fig. 11 a Oil yield and production for the different bioreactors (same symbols as in Fig. 8) separated in to four groups, and **b** the same groups plotted against the bioreactor surface area and volume. The shaded area

represents the range in volumes and surface areas for the closed bioreactors in the group 2 (higher yield and higher production)

criticism for their usage of large amounts of land and water [241, 242]. Their advantage is that they do not require high production rates to produce high oil yields, only large volumes. In contrast, smaller horizontal reactors can conserve water by recycling it; however, they too have a large footprint, and because they have lower volumes (10^4 L), they require higher cell densities to achieve the same high yields as ponds. Ponds and tubular reactors have lower production but higher cell densities than algae growing in unlimited nutrient and light conditions in small (<1 L) laboratory cultures. This may be a result of the infamous “bottle effect” [246], or it may simply be a result of contamination/competition, low light or nutrients, or inefficient mixing. Whatever the reason, it seems that larger cultures are less productive yet more concentrated than those at bench scales. This makes system scaling more unpredictable, and no practical scale-up methods exist although several design parameters have been identified, e.g., light, CO_2 , mixing, etc. [247–257]. Since production decreases with bioreactor volume, smaller bioreactors (10^2 – 10^3 L) may be more practical, especially if they are designed to provide high surface areas for high illumination [257, 258]. Indeed, of 317 bioreactors studies analyzed in this paper, only 8.5 % had production rates $>2.5 \text{ g L}^{-1} \text{ day}^{-1}$, and all of these bioreactors, except for two, were smaller systems with a mean volume of 322 L, but achieved high biomass concentrations and/or growth rates by a combination of high illuminated surface areas (i.e., high SA:V) and moderate mixing with air or 1–5 % CO_2 . Interestingly, while air lift mixing is a cheap and an efficient means to deliver high dissolved inorganic carbon (DIC) levels to cultures and keep cells mixed, bubbles also can have a high attenuation of light and create high shear when they break. Therefore, the interplay between homogenous mixing of biomass to optimize light levels in bioreactors, air mixing to enhance DIC, and high surface to volume ratio of bioreactors needs to be explored in more detail to find the optimal conditions.

Many biofuel bioreactors, such as coils, flat plates, and cascades, have been designed to optimize the surface to volume ratio to provide high-light conditions to cells. This design promotes higher production rates, by enhancing biomass and maintaining higher growth rates. However, high production rates must be linked to high yields, which can be achieved by larger volumes. The caveat is that large volume systems with high surface to volume ratios drastically increase the footprint of the culture system, thereby competing for space with agricultural crop or high biodiversity lands, which is a major criticism of commercial algal processes. Systems with large surface areas also compete for solar radiation with other renewable energy systems, such as photovoltaics and solar water heaters. Other than higher production rates and smaller footprints, there are other advantages to small bioreactors, such as they require less energy-consuming components (e.g., one, small pump instead of many large ones) and smaller downstream-processing units. The later makes harvesting and extracting biomass from a

small closed reactor simpler and less expensive than harvesting raceway ponds [259]. Thus, the combination of less power consumption, fewer materials, and higher biomass and production rates could reduce the price of algal oil, which would be another advantage of smaller bioreactors.

To overcome the problem of low yields in small bioreactor systems, the surface area being irradiated should be optimized for the minimum volume that achieves high production rates ($\geq 1 \text{ g L}^{-1} \text{ day}^{-1}$) by maintaining high algal concentrations (e.g., $\geq 1 \text{ g L}^{-1}$) and growth rates (e.g., 1 day^{-1}). In theory, one hundred thirty, 200-L bioreactors could attain a cumulative oil yield of about 10 L day^{-1} ($1.1 \text{ L oil kg}^{-1} \text{ lipids} \times 0.7 \text{ recovery efficiency} \times 0.5 \text{ lipids} \times 130 \text{ bioreactors} \times 200 \text{ L bioreactor}^{-1} \times 10^{-3} \text{ kg L}^{-1} \text{ day}^{-1}$); the same yield as half of the ponds surveyed in this paper, provided the lipid content of the cells, is around 50 % of the dry weight. The number of bioreactors could be reduced to 33 if production could be maintained at the maximum level of $4 \text{ g L}^{-1} \text{ day}^{-1}$. One approach to increase the irradiated area of a small volume bioreactor would not be to increase the exterior surface area (i.e., footprint), but increase photon flux inside the system. Internal daylighting or a dense distribution of internal lights could achieve this and also reduce the light path. If these were incorporated into a small volume vertical bioreactor, the spatial footprint would be small, minimizing the use of open space without displacing agriculture, biodiversity, or green space. Therefore, designs of future culture systems should pay closer attention to high yields and low footprints [260–265], as well as minimal energy consumption [266]. This would be especially applicable to smaller commercial volumes (i.e., 200 L) with high illuminated areas and adequate mixing of cells through the light field, but not at such high turbulent levels that would increase cell mortality, and thus reduce biomass [267–269].

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