

Effect of silicate limitation on growth, cell composition, and lipid production of three native diatoms to Southwest Texas desert

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Abstract To understand the potential of *Nitzschia* spp. in the biofuels industry, growth characteristics and fatty acid composition of three strains of *Nitzschia* grown at five silicate concentrations were investigated. The algae were grown in two stages with temperatures and light: dark cycles designed to mimic spring/fall conditions in the southern United States. First, cells were grown on Si concentrations ranging from 0.2 to 10.6 mol m⁻³. Second, they were grown for four more days with half of the initial medium replaced by a Si-free medium. Growth rates ranged from 0.16 to 0.30 day⁻¹ in Si media and 0.07–0.39 day⁻¹ in Si-free media. The total lipid content in the Si-free media ranged from 26.6 to 255 mg g⁻¹ AFDW. In all cases, the fatty acid distribution was similar between strains, with palmitic acid (16:0) and palmitoleic acid (16:1)

dominant. The results show that lipid production of native *Nitzschia* is strain-specific, and all three strains are potential candidates for the biofuel industry.

Keywords Bacillariophyceae · Microalgal biofuels · Silicate stress · Growth · Lipid · Cell composition

Introduction

The most common genera used in microalgal biofuel arena at present are from the green algae and diatoms (Chisti 2007; Fields et al. 2014). Diatoms, a group of algae with silica cell coverings and good oil production capability, produce lipids (e.g., triacylglycerols, TAGs) and carbohydrates as their main storage compounds (Becker 2004), thus holding promise as feedstock for biodiesel (Kyle and Gladue 1991). Phytoplankton cells undergoing nutrient limitation induce metabolic acclimatization, which often results in marked changes in their biochemical and fatty acid composition. For example, silicate (Si) limitation leads to lipid accumulation in the diatoms *Chaetoceros graclis*, *Hantzschia* sp., and *Cyclotella* sp. (Roessler 1988a; Taguchi et al. 1987), while nitrogen (N) limitation leads to lipid accumulation in *Dunaliella tertiolecta* and *Thalassiosira pseudonana* (Jiang et al. 2012) and many other algae (Griffiths et al. 2012). Si-starved *T. pseudonana* accumulated an average of 24 % more TAG than those starved for N, though the chemotypes of the TAGs produced were generally similar (Yu et al. 2009).

Nitzschia spp., like other diatoms, have simple nutritional requirements, fast growth rates, and high lipid production (Chagoya et al. 2014; Jiang et al. 2014). Native strains were chosen in anticipation of a more successful production of biomass and high oil content, which can lead the way for economical large-scale production of microalgal biofuel even in harsh climates (Chagoya et al. 2014; Jiang et al. 2014). The

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need for local microalgae strains that can be easily mass cultured and are suited to the local environment remains an issue. Besides species selection, cultivation, and nutrient conditions, the other requirements that must be considered before growing photoautotrophic microalgae with either open or closed cultivation systems for microalgae-derived biofuels include adequate solar radiation and suitable climate and temperatures (Chisti 2007; Sheehan et al. 1998; Spolaore et al. 2006).

The aims of this study were compare the growth, lipid production, and fatty acid composition of three strains of *Nitzschia* isolated and then cultivated under spring/fall conditions typical in the southern states of the United States, with the objective to determine their feasibility for biofuel production in this region, and to improve biomass and lipid production for biofuel production. Some of the findings for *Nitzschia* strain LBK-017 have been published previously (Jiang et al. 2014); they are included here for comparative purposes.

Materials and methods

Native *Nitzschia* strains TAMU-LBK-017 and TAMU-LBK-018 were isolated from a seasonal playa lake in Lubbock, Texas, USA, by streaking onto a series of agar plates prepared with artificial growth phase media (see below). *Nitzschia perspicua* Cholnoky was isolated by General Atomics (San Diego, California, USA). The isolates were identified morphologically to genus level by observation of cleaned cells using light microscopy. Subsequent DNA sequencing (data not shown) did not help to determine the species identity. TAMU-LBK-018 and TAMU-LBK-017 will be referred to as LBK-018 and LBK-017, respectively, herein.

The temperature used to grow the diatoms varied from 10 to 25 °C; cycles were chosen to mimic those typically experienced during the spring/fall periods in Midland, Texas (Table A1; for more information see Jiang et al. (2014)). All cultures were grown in triplicate 1 L polycarbonate bottles following the method of Jiang et al. (2014). In all cases, diatoms were acclimated to the treatment conditions (temperatures, light: dark cycle and medium) for ~10 generations before starting experiments. An artificial brackish growth medium was developed using groundwater from Midland, Texas (Chagoya et al. 2014; Jiang et al. 2014), to which nutrients (N, P), trace elements, and vitamins were added as well as NaHCO₃ as a carbon source. *Nitzschia* spp. were grown with five Si concentrations (0.2, 1.1, 2.1, 4.2, and 10.6 mol m⁻³ Si). Measurements of diatom characteristics were made during the exponential phase; referred to as growth phase (GP). After cultures reached late exponential and before the stationary phase, diatom cultivation was continued into lipid formation phase (LP), that is, half of the volume of culture was harvested and replaced with fresh medium lacking

Si but with the same N and P concentrations. The incubation duration for *N. perspicua* was 3 days in both GP and LP; for LBK-018, GP was 3 days and LP was 5 days; for LBK-017, GP and LP were both 4 days. Cultures were monitored as during the GP. For simplicity, we will refer to the LP treatments by the corresponding GP Si concentrations.

Analytical procedures

Algal growth was monitored by daily changes in OD₇₅₀ (UV-2501PC UV-VIS, Shimadzu) following the method of Jiang et al. (2014). Growth rates (μ , day⁻¹) were calculated as $\mu = 1/T (\ln N_{t+1} - \ln N_t)$ day⁻¹, where N_t is the OD₇₅₀ reading at time point t , N_{t+1} is the OD₇₅₀ reading at time point $t+1$, and T (days) is the interval between observations.

Dry weight (DW) and ash-free dry weight (AFDW) were measured following the protocols of Jiang et al. (2014). In brief, algal cultures were filtered onto precombusted (500 °C, 2 h) glass fiber filters (Whatman GF/F, 47 mm) and rinsed with a solution of 0.335 N NaCl in 0.1 N HCl, followed by a rinse with 0.435 M ammonium formate to remove the NaCl. Samples were dried to constant weight at 103–105 °C (at least 2 h) to determine DW, then ashed at 500 °C for 2 h to determine AFDW. Ash content was expressed as the percentage of ash weight in DW. For carbon and nitrogen content, samples (300–500 mL) were collected by centrifugation (4,000×g, 15–20 min). The algae pellets were washed with isotonic sodium chloride solution (0.26 N NaCl) three times, and the NaCl was removed by briefly rinsing in DI water. Harvested cultures were lyophilized and analyzed with a CHN analyzer (PerkinElmer 2400 CHNS/O Analyzer). Acetanilide was used as external standard.

The changes of intracellular neutral lipid concentration during the LP experiment was followed by changes in fluorescence intensity of Nile Red (NR) stained cultures as described previously (Jiang et al. 2014). The relative NR fluorescence intensity is then normalized to cell density using OD₇₅₀ as lipid index (lipid index = total area calculated under 580 nm peak/absorbance measured at 750 nm).

For fatty acid analysis, the culture sample was filtered onto precombusted 47 mm Whatman GF/F membranes (500 °C, 1 h). Filters were stored in the -20 °C freezer until analysis. Fatty acid methyl esters (FAMES) were prepared by a modified acid transesterification with H₂SO₄-methanol (4 %) for 1 h at 99 °C with continuous mixing for producing FAMES (Revellame et al. 2010). The FAME composition was determined using an Agilent 7890A gas chromatograph (GC) and 5975C mass spectrometer (MS) (Agilent) with automatic injector (Agilent 7683B automatic liquid sampler) and DB-WAX capillary column (30 m×0.25 mm id). The quantities of individual FAMES were identified and estimated from the retention time and peak areas on the chromatogram using authentic standards (Nu-Chek-Prep reference standard mix,

GLC-68A). More details of these methods are given in Jiang et al. (2014).

The iodine value ($\text{g I}_2 (100 \text{ g})^{-1}$) was calculated from ester content according to Annex B of European biodiesel standard EN 14214 (UNE-EN 14214 2003). It is calculated in gram of iodine which react with 100 g of the respective sample when formally adding iodine to the double bonds. Biomass and lipid productivity during LP was calculated as revised from the equation of Huerlimann et al. (2010). Specific growth rate (μ , day^{-1}) and dry weight (DW, g L^{-1}) were used to calculate biomass productivity (P_{DM}): $P_{\text{DM}} = \text{DM} \times \mu \times 1000$ ($\text{mg L}^{-1} \text{ day}^{-1}$). Lipid content (LC, total lipid% of DW) and biomass productivity (P_{DM} [$\text{mg L}^{-1} \text{ day}^{-1}$]) were used to calculate lipid production (P_{L}) according to the equation $P_{\text{L}} = P_{\text{DM}} \times \text{LC} / 100 \%$ ($\text{mg L}^{-1} \text{ day}^{-1}$).

Statistical analysis

A three-way ANOVA analysis was performed on the growth rate, AFDW, DW, and ash% of DW for each treatment to assess the effects of phases, treatment, and strain on growth and biochemical response. Because multiple three-way ANOVA interactions occurred, the differences among the five treatments and three strains were also investigated by conducting a one-way ANOVA with LSD post hoc tests for each treatment. p values of <0.05 were used as standard for statistical significance (SPSS, 20.0).

Results

Growth rates under different Si concentrations during GP and LP

Growth rates of three *Nitzschia* strains were significantly different among Si concentrations during GP (*N. perspicua*: $F_{4,10} = 6.074, p < 0.01$; LBK-018: $F_{4,10} = 3.724, p < 0.05$; LBK-017: $F_{4,10} = 6.609, p < 0.01$, One-way ANOVA). The highest growth rates for all strains were measured in the 4.2 mol m^{-3} Si treatments (between 0.23 ± 0.01 and $0.30 \pm 0.03 \text{ day}^{-1}$, Fig. 1a), with significant difference against other treatments only for LBK-017 (LSD, $p < 0.05$, with exception of 2.1 mol m^{-3}). It appears that the highest concentration of Si used (10.6 mol m^{-3}) may have been toxic, inhibiting diatom growth, given the growth rates were typically lower than those measured when 4.2 mol m^{-3} Si was used. Three-way ANOVAs showed that the interactions among phase, Si, and strain were significant for growth rate of *Nitzschia* spp. ($p < 0.01$, except for phase \times treatment) (Table 1).

After transfer into Si-free medium, cells continued to grow with rates ranging from 0.07 ± 0.03 to $0.39 \pm 0.0 \text{ day}^{-1}$ (Fig. 1b). Growth rates of three *Nitzschia* strains were significantly

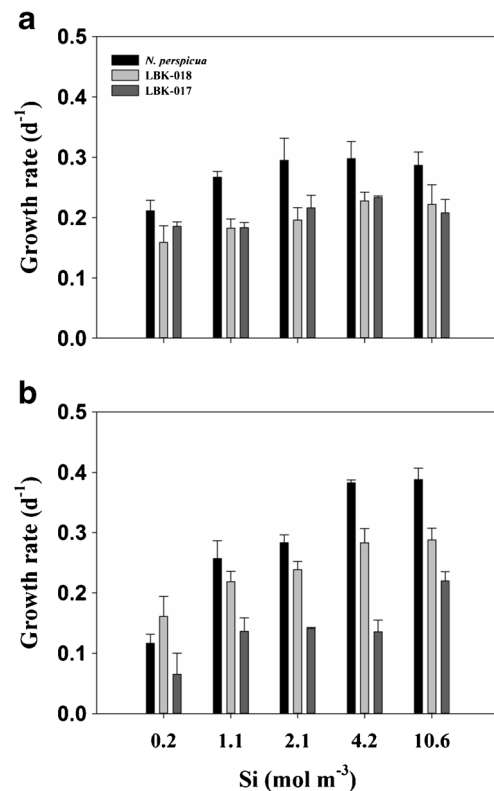


Fig. 1 Growth rates (day^{-1}) of *Nitzschia* spp. cultivated in different Si concentrations during **a** growth phase (GP) and **b** lipid formation phase (LP). Error bars represent standard deviations ($n=3$). If the error bars are not visible, they are smaller than the symbol

different with Si concentrations (*N. perspicua*: $F_{4,10} = 112.07, p < 0.001$; LBK-018: $F_{4,10} = 15.14, p < 0.001$; LBK-017: $F_{4,10} = 17.01, p < 0.001$). Growth rate of LBK-017 in medium containing 0.2 mol m^{-3} Si was the lowest compared to other Si treatments (LSD, $p < 0.05$). Growth rate in the lipid formation media were compared to Si concentration, assuming other nutrients were saturating, and did produce a statistically significant fit to the logarithm regression ($p < 0.0001$, Table 2). Coefficients of determination (r^2) ranged from 0.746 to 0.933 for growth rate as a function of Si concentration.

Changes in biochemical composition under different Si concentrations during GP and LP

DW, AFDW, and C/N ratios of the three strains in the five concentrations of Si are shown in Table 3. During GP, the maximum biomass (DW) achieved were: *N. perspicua*, $0.31 \pm 0.11 \text{ g L}^{-1}$ in 1.1 mol m^{-3} Si medium (LSD, $p < 0.05$); LBK-018 and LBK-017, $0.24 \pm 0.01 \text{ g L}^{-1}$ in 10.6 mol m^{-3} Si medium (LSD, $p < 0.001$). The highest DW at the end of lipid formation phase was $0.62 \pm 0.15 \text{ g L}^{-1}$ in the treatments with 2.1 mol m^{-3} Si for *N. perspicua*; four times more than measured in the corresponding GP media. For LBK-018, the highest DW was $\sim 0.34 \text{ g L}^{-1}$ in the 2.1, 4.2, and

Table 1 Three-way ANOVA on the effect of phase, treatment, and strain on the growth and biochemical compositions of *Nitzschia* spp. with spring/fall condition. Phase: growth phase (GP) and lipid phase (LP);treatment 0.21, 1.1, 2.1, 4.2, and 10.6 mol m⁻³ Si; strains: *N. perspicua*, LBK-018, LBK-017; phase, treatment, and strain were treated as fixed variables

Source of variation	Growth rate	AFDW	DW	Ash% DW	C% DW	N% DW	C/N ratio
Phase	NS	***	***	***	**	NS	NS
Treatment	***	***	***	**	***	***	***
Strain	***	***	***	***	NS	NS	***
Phase × treatment	***	***	***	***	NS	**	**
Phase × strain	***	*	*	NS	**	NS	**
Treatment × strain	***	*	**	NS	NS	NS	**
Phase × treatment × strain	***	NS	**	***	NS	NS	***

NS not statistically significant at least at $p < 0.05$ level* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

10.6 mol m⁻³ Si treatments while it was 0.49±0.04 g L⁻¹ in the 4.2 mol m⁻³ Si for LBK-017 (Table 3).

Ash content of three strains of *Nitzschia* ranged between 9.9±2.9 and 73.8±0.87 % of DW during GP, increasing with increasing Si in the media (Fig. 2). The highest overall ash contents during GP were in 10.6 mol m⁻³ Si medium: LBK-018 (73.8±0.87 % DW), *N. perspicua* (51.6±9.15 % DW), and LBK-017 (66.1±2.13 % DW), which were all higher than other treatments (LSD, $p < 0.05$, Fig. 2). Ash content during LP showed a similar trend in responding to the Si concentrations in the medium as in GP (Fig. 2). LBK-018 had a steeper slope of 4.21 ($r^2 = 0.95$) between the ash content and Si concentrations in the media than that of LBK-017 (slope=3.60, $r^2 = 0.76$) and *N. perspicua* (slope=3.28, $r^2 = 0.79$) indicating that it responded more strongly in changing cellular ash content.

AFDW during GP ranged from 0.04±0.01 to 0.25±0.1 g L⁻¹ with strain differences in five Si concentration media (Table 3). Results of three-way ANOVAs revealed that phase, treatment, and strain had a significant effect ($p < 0.001$) on DW, AFDW, and ash content (Table 1). AFDW was two to five times higher in *N. perspicua* grown in 0.2 and 1.1 mol m⁻³ Si medium than for LBK-017 or LBK-018. Highest AFDW in the lipid phase were found in strains and treatments comparable to those for DWs (Table 3).

To further study the effect of Si concentrations on biomass production, cellular carbon, and nitrogen were measured, and C/N molar ratios of three strains under five Si concentrations were compared (Fig. 3; Table 3). During GP and LP, the C and

N% of DW of all three strains were generally higher when growing at lower Si concentrations (Fig. 3). Highest C and N% of DW were measured at 0.2 mol m⁻³ Si for all three strains during GP and LP (LSD, $p < 0.05$, except for LBK-017 in LP). During GP, the highest C/N ratios were achieved in 10.6 mol m⁻³ Si medium for LBK-018 and LBK-017, with significant differences to other Si treatments (LSD, $p < 0.001$), achieved in the lowest Si medium for *N. perspicua* with no significant differences from 4.2 and 10.6 mol m⁻³ Si (LSD, $p = 0.837$ and $p = 0.13$, respectively). The highest C/N ratios during the LP were measured in the 0.2 mol m⁻³ Si treatment in all three strains and ranged from 10.5±1.62 to 15.2±0.2 (Table 3).

Lipid formation under different Si concentrations during LP

The NR fluorescence assay was used to determine relative lipid index (content) daily after the transfer from GP to LP media. By the 2nd–4th day after transfer, NR fluorescence indicated intracellular neutral lipid bodies increased to the maximum values for all three strains, and diatom cells that were most Si stressed showed the highest values (Fig. 4). *N. perspicua* and LBK-018 accumulated more lipid at lower Si concentrations, while LBK-017 appeared to accumulate more lipid at the highest Si concentration (Fig. 4).

Total lipid ranging from ~27–255 mg g⁻¹ AFDW varied with the gradient of Si concentrations in the media and strains (Table 4). As supported by the lipid content measured by NR fluorescence method, cultures of *N. perspicua* and LBK-018 accumulated significantly higher amounts of lipid, 159 and 255 mg g⁻¹ AFDW, respectively, in the most Si stressed medium (0.2 mol m⁻³ Si) than other Si concentration media (LSD, $p < 0.05$, Fig. 4). The fatty acid profiles, as shown in Table 4, indicated the presence of C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C20:4, and C20:5. Among the fatty acids measured in the three strains of *Nitzschia*, palmitic acid (C16:0; saturated fatty acid, SFA) and palmitoleic acid (C16:1; monounsaturated fatty acid, MUFA) were found to

Table 2 Coefficients of determination (r^2) and significance for logarithmic models of algal growth rates (μ) as a function of silicate concentrations during lipid formation phase ($n = 15$)

Strain	r^2	Significance
<i>N. perspicua</i>	0.933	<0.0001
LBK-018	0.834	<0.0001
LBK-017	0.746	0.0001

Table 3 Changes in the biochemical compositions of *Nitzschia* spp. with growth and lipid formation phases. Results are expressed as mean±standard deviation (SD, n=3)

	Treatment									
	GP					LP				
Si (mol m ⁻³)	0.2	1.1	2.1	4.2	10.6	0.2	1.1	2.1	4.2	10.6
Dry weight (g L ⁻¹)										
<i>N. perspicua</i>	0.25±0.1	0.31±0.11	0.15±0.01	0.17±0.04	0.18±0.01	0.25±0.06	0.34±0.13	0.62±0.15	0.46±0.03	0.4±0.07
LBK-018	0.08±0.01	0.1±0.04	0.11±0.02	0.07±0.01	0.24±0.01	0.2±0.01	0.27±0.02	0.33±0.05	0.34±0.02	0.34±0.01
LBK-017	0.14±0.02	0.14±0.01	0.14±0.01	0.18±0.01	0.24±0.02	0.13±0.01	0.27±0.01	0.37±0.06	0.49±0.04	0.32±0.06
AFDW (g L ⁻¹)										
<i>N. perspicua</i>	0.22±0.09	0.25±0.1	0.09±0.01	0.11±0.02	0.09±0.02	0.2±0.05	0.29±0.11	0.47±0.14	0.32±0.03	0.25±0.04
LBK-018	0.05±0.01	0.05±0.01	0.05±0.005	0.04±0.01	0.06±0.002	0.14±0.01	0.19±0.02	0.2±0.02	0.19±0.02	0.14±0.01
LBK-017	0.11±0.01	0.09±0.004	0.09±0.004	0.12±0.01	0.08±0.004	0.12±0.05	0.22±0.003	0.27±0.04	0.31±0.05	0.17±0.05
C/N ^a										
<i>N. perspicua</i>	10.4±0.6	8.54±0.56	8.6±0.4	10.3±0.52	9.78±0.27	15.2±0.2	11±0.24	9.12±0.11	9.47±0.65	10.4±0.68
LBK-018	9.21±0.26	8.98±0.15	9.16±0.23	9.8±0.12	14.8±0.29	14.3±1.11	10.9±0.31	9.92±0.47	10.5±1.25	10.3±0.51
LBK-017	9.65±0.69	8.5±0.01	8.46±0.46	9.09±0.29	11.5±0.17	10.5±1.62	9.34±0.46	8.98±0.81	8.53±0.27	10.3±1.6

GP growth phase, LP lipid formation phase under Si limitation

^a C/N ratios are calculated on molar basis

be the main components, which ranged from 12.4±0.2 to 25±0.7 % and 39.6±0.03 to 60.3±1.8 % of total FAME, respectively, whereas the myristic acid, linoleic acid, stearic acid, oleic acid, eicosapentaenoic acid, and arachidonic acid were present as minor fatty acids only (Table 4). The proportion of SFA and MUFA decreased with the increasing Si concentration (Fig. 5). For all three *Nitzschia* strains, MUFAs (on average of 48.4±7.3 % for *N. perspicua*, 55.8±3.9 % for LBK-018, and 47.2±4.9 % for LBK-017, n=15) were the most abundant relative to SFAs and polysaturated fatty acids (PUFAs) (Fig. 5). For *N. perspicua* and LBK-018, SFA and

MUFA showed a gradient of decreasing concentrations with increased Si concentrations; however, PUFA increased with the increase of Si concentrations (Fig. 5a, b).

One of the common parameters used to estimate the quality of biodiesel is the iodine value, which is a measure of the degree of unsaturation of the mixture of fatty acid sample. In this study, the change in Si concentrations led to a change in the strain-specific iodine values (Table 4). The highest iodine values ranged from 60 (*N. perspicua* and LKB018 in 0.2 mol m⁻³ Si medium) to 69 (LBK-017 in 2.1 mol m⁻³ Si medium), and the lowest iodine values ranged from 47 (*N. perspicua* in 4.2 mol m⁻³ Si media and LBK-017 in 0.2 mol m⁻³ Si medium) to 53 (LBK-018 in 4.2 mol m⁻³ Si media) (Table 4).

Biomass productivity increased with the increased Si concentrations for LBK-018 (53.0±5.87 mg L⁻¹ day⁻¹ during GP; 96.8±5.92 mg L⁻¹ day⁻¹ during LP) and LBK-017 (49.4±8.67 mg L⁻¹ day⁻¹ during GP; 70.2±11.0 mg L⁻¹ day⁻¹ during LP) (Table 5). The most biomass productivity for *N. perspicua* was 84.5±31.9 and 176±14.4 mg L⁻¹ day⁻¹ during GP and LP, respectively, 1.7 and 2.5 times higher than those of LBK-017, respectively. The most lipid productivity for *N. perspicua* and LBK-018 was ~8–9 mg L⁻¹ day⁻¹, which was significantly higher than the most lipid productivity of LBK-017.

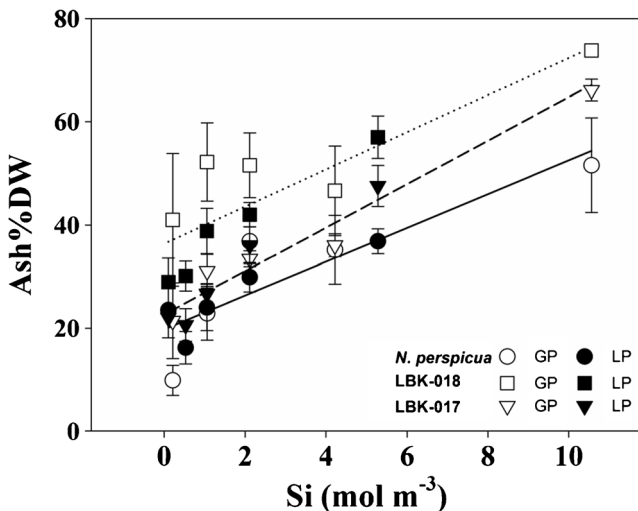
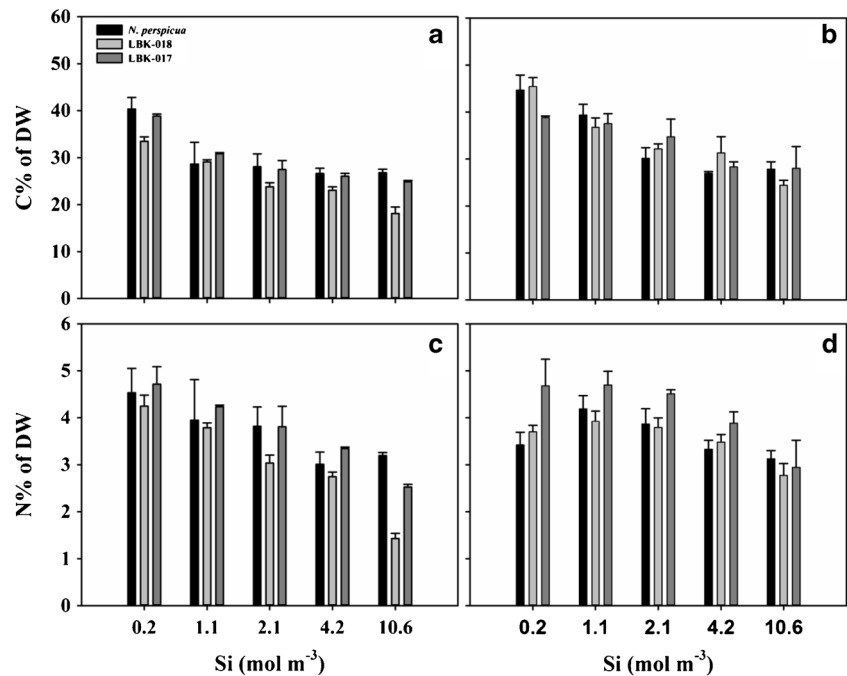


Fig. 2 Effect of Si concentrations on the percentage of ash (% DW) of *N. perspicua* (solid line, circles), LBK-018 (dot line, squares), and LBK-017 (dash line, triangles) in the GP (open symbols) and LP (solid symbols). Error bars represent standard deviations (n=3). If the error bars are not visible, they are smaller than the symbol

Discussion

Si, as a key macronutrient element for diatom growth, has significant effects on their growth rates when supplied with

Fig. 3 Cellular carbon (C) and nitrogen (N) content % of DW in the GP (a, c) and LP (b, d) of *Nitzschia* spp. Error bars represent standard deviations ($n=3$). If the error bars are not visible, they are smaller than the symbol



sufficient N and P. Results in this study showed that low levels of Si reduced diatom growth in most cases, and the relationship between growth rate and Si fit for nonlinear model, which varied slightly among strains (Fig. 1; Table 2). The growth rates measured are in the range previously reported for other diatoms (Jiang et al. 2012; Roessler 1988a; Taguchi et al. 1987; Yu et al. 2009) suggesting that the spring/fall conditions allowed relatively high growth rates. Other studies examining the effect of Si limitation on growth have found no significantly different growth rates (Chu et al. 1996; Parslow et al. 1984; Taguchi et al. 1987). Two explanations can be used to account for differences between our data and previous studies. First, we used half the initial concentration of Si in the lipid phase, whereas previous studies have utilized much lower Si media (2–176 mmol m⁻³ Si) (Chu et al. 1996; Parslow et al.

1984; Taguchi et al. 1987). Second, we followed Si limitation for only 4 days since our primary interest was stimulating lipid accumulation rather than investigating more serious consequences of nutrient limitation on diatom physiology. Hence, when comparing studies, these two factors need to be considered.

Dry weight is an important parameter for estimating biomass concentration, productivity, and percentage of cell components, including both inorganic (e.g., minerals) and organic (e.g., sugars, lipids, proteins, and nucleic acids) contents of the sample, and the residual ash includes most of the inorganic content. In the study conducted by Chu et al. (1996), the authors found that the Si levels (8.8–176 mmol m⁻³ Si) did not significantly affect the growth and AFDW of *N. inconspicua*, which was not the same as what we found in this study. A possible explanation is the higher overall Si concentrations used in this study. Si is known to affect biomass productivity and lipid production in many algae (Roessler 1988a; Taguchi et al. 1987; Tyrrell and Law 1997; Yu et al. 2009), but very little literature exists regarding the effect of Si concentrations on DW. Normalization to DW is inadequate for evaluating the biofuels production when manipulating cultivation conditions to achieve a better production on oil or byproducts.

Ash or inorganic content in biomass may account for 27–55 % DW of diatoms and 5.3–19.9 % DW of planktonic chlorophytes (Nalewajko 1966) and depends on the species and the conditions it grows in. Except the higher ash content values under the highest concentration of Si, most ash contents of *Nitzschia* in this study are comparable with those reported for other species including: *Nitzschia inconspicua*,

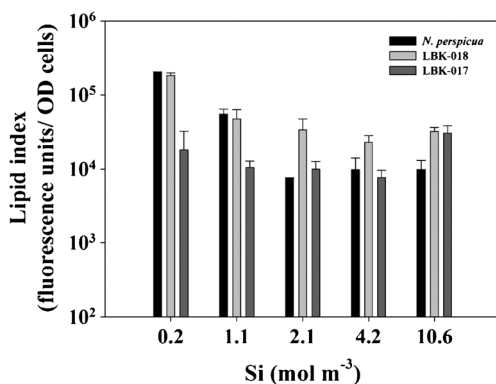


Fig. 4 The neutral lipid content stained with Nile Red (NR) per OD₇₅₀ unit at the harvest day in LP of *N. perspicua* (Day 3), LBK-018 (Day 5), and LBK017 (Day 4). Error bars represent standard deviations ($n=3$). If the error bars are not visible, they are smaller than the symbol

Table 4 Fatty acid composition of *Nitzschia* spp. grown in medium with different silicate concentrations. Results are expressed as mean±standard deviation (SD, *n*=2 or 3 depending on presence/absence of lipid)

Fatty acid	0.2 mol m ⁻³ Si			1.1 mol m ⁻³ Si			2.1 mol m ⁻³ Si			4.2 mol m ⁻³ Si			10.1 mol m ⁻³ Si		
	<i>N.perspicua</i>	LBK018	LBK017	<i>N.perspicua</i>	LBK018	LBK017	<i>N.perspicua</i>	LBK018	LBK017	<i>N.perspicua</i>	LBK018	LBK017	<i>N.perspicua</i>	LBK018	LBK017
C14	6.8±0.06	7.12±0.46	3.41±0.37	4.97±0.3	5.83±0.77	3.45±0.48	4.28±0.02	6.24±0.1	3.65±0.48	4.84±0.2	6.46±0.33	3.15±0.24	5.17±0.11	9.4±0.57	6.09±0.65
C16:0	22.7±0.43	24.0±0.17	21.8±0.37	22.2±2.34	25.2±0.71	15.5±0.3	16.9±0.46	20.0±0.46	12.4±0.17	16.6±0.99	20.2±0.47	14.1±0.49	17.1±1.35	17.4±0.33	16.7±2.04
C16:1 n-7	58.9±0.07	60.3±1.8	40.4±2.6	50.2±1.24	56.2±1.63	45.6±2.97	39.6±0.03	53.5±2.76	45.8±1.36	41.4±1.18	50.6±2.67	43.2±1.17	45.8±1.95	53.1±0.99	53.0±3.33
C16:2 n-2			5.65±0.44	3.61±0.73		4.55±0.75	5.0		4.42±0.71	6±0.06	0.9	5.55±0.37		3.48±0.93	
C16:3 n-3	1.83±0.11		4.11±0.42	4.52±0.93		5.59±0.4	10.2±0.71		6.87±0.66	9.74±0.75	0.4	6.55±0.64	8.57±1.18		3.73±0.97
C18:0	0.7±0.05	1.58±1.71	3.67±2.68	0.7±0.07	1.93±0.24	0.68±0.08	0.92±0.08	2.07±0.91	1.05±0.38	0.84±0.17	1.53±0.6	0.43±0.03	0.8±0.28	1.93±0.19	0.88±0.51
C18:1 n-9	1.27±0.04	1.77±0.97	1.4	1.49±0.18	1.45±0.36	1.23±0.16	1.26±0.13	1.0	1.97±1.34	1.13±0.12	1.71±1.09	0.98±0.14	1.15±0.01	1.23±0.05	1.21±0.14
C18:2 n-6	1.64±0.4	2.0	4.51±0.7	3.37±0.95		9.55±1.89	4.81±0.75	5.7±1.71	13.5±0.52	3.67±0.31	5.1	7.39±1.92	4.73±0.16	4.5	8.05±0.37
C18:3 n-6						0.5			0.7			0.71±0.07			0.41±0.1
C20:2 n-6						0.6			0.69±0.25						
C20:4 n-6	2.44±0.25	1.94±0.08	7.21±0.04	5.68±0.6	5.57±0.47	5.62±1.55	10.7±0.45	6.46±1.48	4.17±0.53	7.25±0.54	7.29±0.55	5.58±0.48	5.86±0.44	6.1±0.2	2.31±1.31
C20:5 n-3	2.3±0.14	2.55±0.75	8.61±0.14	3.47±0.84	4.97±0.17	6.88±2.3	5.6±0.82	7.92±1.2	4.51±0.44	8.83±0.76	9.26±1	11.0±1.01	6.54±0.31	10.4±0.55	3.96±2.28
C24:0					0.3		1.43±0.08	1.8±0.25	1.1	1.1±0.98		1.99±0.52	2.35±0.31	2.1	1.5
Total lipids (mg g ⁻¹ AFDW)	159±51.2	255±13.2	45.8±2.78	107.5±2.4	104±3.49	58.3±4.34	26.6±7.53	75.5±17.1	40.4±0.43	36.7±4.8	75.3±10.2	41.4±7.19	54.7±4.04	90.3±13.4	91±16.8
Iodine value	59.9±0.74	59.9±1.44	46.8±0.39	54.8±0.3	54.2±1.99	61.4±5.8	47.0±1.4	57.7±3.6	69.2±1.4	46.6±0.8	52.5±3.5	56.5±4.6	52.7±1.6	53.7±2.9	66.4±3.6

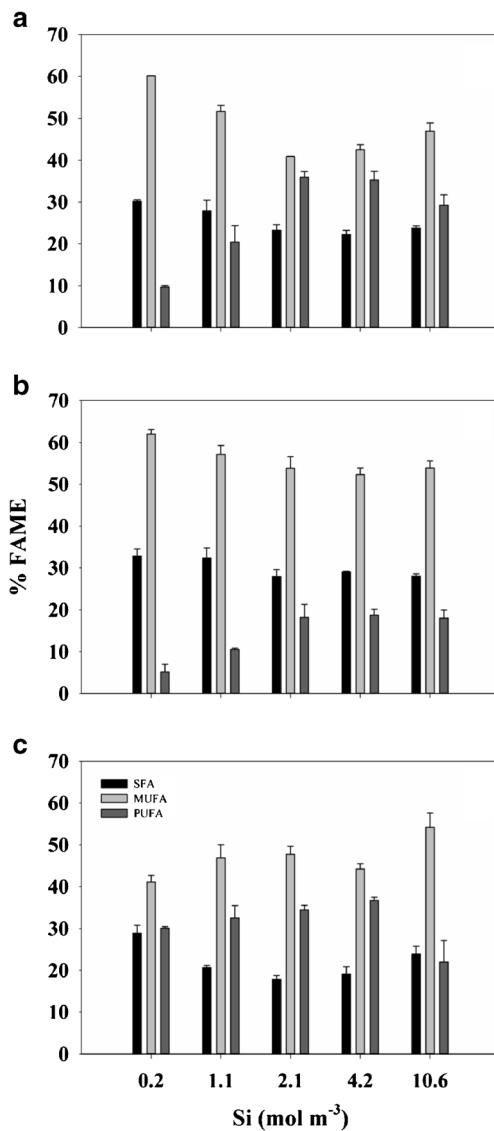


Fig. 5 The percentages of saturated fatty acid (SFA, black), monounsaturated fatty acid (MUFA, white), and polyunsaturated fatty acid (PUFA, grey) in total fatty acids of *N. perspicua* (a), LBK-018 (b), and LBK-017 (c) under the spring/fall condition at the end of LP. Error bars represent standard deviations ($n=3$). If the error bars are not visible, they are smaller than the symbol

Table 5 Biomass and lipid productivity ($\text{mg L}^{-1} \text{day}^{-1}$) of *Nitzschia* spp. based on dry weight grown in lipid formation media with different Si concentrations. Results are expressed as mean \pm standard deviation (SD, $n=3$)

Treatment	Biomass productivity ($\text{mg L}^{-1} \text{day}^{-1}$)						Lipid productivity ($\text{mg L}^{-1} \text{day}^{-1}$)		
	GP			LP			LP		
Si (mol m^{-3})	<i>N.perspicua</i>	LBK-018	LBK-017	<i>N.perspicua</i>	LBK-018	LBK-017	<i>N.perspicua</i>	LBK-018	LBK-017
0.2	53.9 \pm 25.6	13.3 \pm 3.17	26.3 \pm 4.28	29.1 \pm 2.79	32.5 \pm 8.18	11.57 \pm 8.2	4.55 \pm 1.06	8.36 \pm 2.54	-
1.1	84.5 \pm 31.9	18.8 \pm 8.38	24.8 \pm 2.57	86.4 \pm 27.5	59.9 \pm 8.36	37.2 \pm 7.73	7.71 \pm 0.26	6.25 \pm 1.01	2.19 \pm 0.59
2.1	42.9 \pm 6.44	20.5 \pm 7.01	30.2 \pm 1.51	174 \pm 41.1	78.0 \pm 12.7	52.7 \pm 9.0	4.41 \pm 0.06	5.83 \pm 1.32	2.13 \pm 0.34
4.2	49.6 \pm 9.16	15.9 \pm 3.70	41.8 \pm 1.90	176 \pm 14.4	95.5 \pm 11.5	65.8 \pm 10.3	6.31 \pm 1.82	7.11 \pm 0.17	2.73 \pm 0.69
10.6	50.9 \pm 5.44	53.0 \pm 5.87	49.4 \pm 8.67	156 \pm 28.2	96.8 \pm 5.92	70.2 \pm 11.0	8.44 \pm 0.98	8.70 \pm 0.96	6.27 \pm 0.32
Average	56.4 \pm 16.3	24.3 \pm 16.3	34.5 \pm 10.7	124 \pm 64.5	72.5 \pm 26.9	47.5 \pm 23.9	6.31 \pm 1.82	7.25 \pm 1.26	3.33 \pm 1.98

– no data available

from 33.3 to 46.7 % DW and *Nitzschia closterium*, 21.6 % DW (Chu et al. 1996; Zhu and Lee 1997). It appeared that LBK-018 had a greater capability to take up and use silicate than the other two *Nitzschia* spp. in this study, which is supported by the higher ash content values and lower C and N% of DW of LBK-018 under all Si concentrations (Fig. 2; Table 3). A dependence of the Si uptake rate on the external Si concentration has been shown in several studies to approximate the Michaelis-Menten equation (Brzezinski and Nelson 1996; Thamatrakoln and Hildebrand 2008). The increasing ash content (% DW) with increasing Si concentration in both GP and LP media suggests the importance of considering the contribution of ash content to DW when evaluating the biomass production in the biofuels industry.

Our C/N ratios fall in the range reported previously for diatoms. Brzezinski (1985) reported C/N ratios ranging from 4.1 to 29.7 for 28 species grown under 18:6 light dark cycle and 6.2 to 13.3 for 10 species grown under continuous illumination. The higher values of C/N ratios at the end of LP than GP may be caused by the decrease of intracellular N content and the increase of intracellular carbon storage under Si limitation. LBK-018 cells grew slowly and also accumulated lower organic contents (DW, C and N% of DW, Table 3) compared with the other two strains, indicating the lower need for some cellular components.

Lipid formation under different Si concentrations during LP. Oil contents of ~20–50 % of dry weight are quite common (Chisti 2007; Jiang et al. 2012; Roessler 1988a; Sheehan et al. 1998; Taguchi et al. 1987; Yu et al. 2009). Circumstances like principal nutrient starvation (e.g., N, Si, C, and P) can result in marked changes in the biochemical composition and fatty acid composition of diatoms and have been demonstrated as “lipid triggers” for lipid accumulation of algal cells. For instance, under nitrogen limitation, oil content in *Dunaliella tertiolecta* and *Thalassiosira pseudonana* varied between 20 and 25 % (Jiang et al. 2012). Diatoms respond to depleted Si by accumulating lipid because of their dependence on Si to form their frustules (cell walls composed of amorphous silica) (Roessler

1988b; Taguchi et al. 1987; Wen and Chen 2000). For example, *Cyclotella cryptica* increases its percentage of carbon partitioning into lipid by two-fold with higher levels of neutral lipids and higher proportions of SFA and MUFA, whereas that channeled to carbohydrates decreases under Si starvation (Roessler 1988a, b). Coombs et al. (1967) reported that the lipid content of *Navicula pelliculosa* increased by about 60 % after 14-h Si starvation. The reasons of lipid accumulation in response to Si stress have been demonstrated to be an increase in the partitioning of the newly assimilated carbon into the total lipid fraction and also a slow conversion of previously assimilated carbon from non-lipid compounds into lipids (Roessler 1988b). Hence, amending Si concentration in the media appears to be an attractive procedure to increase lipid content in diatoms and improve the iodine values to meet the standard specification according to the European biodiesel standards.

The composition of the fatty acids in the biodiesel has important effects on the quality of biodiesel and the processes required to achieve a fuel for used in ground transportation and aviation. It will be necessary to assess the fatty acid composition and the degree of unsaturation extracted from algal culture to determine the fuel quality achieved. Algal lipids contain a major proportion of MUFA and PUFA and mostly neutral lipids, which makes microalgal lipids as potential replacement for traditional fossil fuel. Our findings suggest that *Nitzschia* spp. produce the oil highly sought after biodiesel production with mostly long chain (C16–22) fatty acids and a high content of C16:1 and C16:0.

The costs for microalgal biomass production could be reduced by significantly increasing biomass productivity and utilizing all the biomass as efficiently as possible. Other high value coproducts or byproducts, such as pigments (e.g., β -carotene, chlorophyll, and phycobiliproteins), vitamins, PUFA, and other specialty chemicals have been considered to lower the cost and enhance the potential economics base of microalgae as a source for biofuels. With the selection of most efficient and robust native microalgal strains, amending medium concentrations and production efficient season condition, it is promising to achieve a better yield in the mass production for the microalgal biofuel industry.

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References

- Becker W (2004) Microalgae in human and animal nutrition. In: Richmond A (ed) Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Science, Oxford, pp 312–351
- Brzezinski MA (1985) The Si:C:N ratio of marine diatoms: interspecific variability and the effect of some environmental variables. J Phycol 21:347–357
- Brzezinski MA, Nelson DM (1996) Chronic substrate limitation of silicic acid uptake rates in the western Sargasso Sea. Deep-Sea Res Part II 43:437–453
- Chagoya J, Brown J, Gomez M, Zhang J, Jiang Y, Lavery K, Brown L, Quigg A, Burow M (2014) Media optimization and lipid formation of two native diatoms for cultivation in the Southwest Texas desert. J Appl Phycol. doi:10.1007/s10811-014-0238-1
- Chisti Y (2007) Biodiesel from microalgae. Biotechnol Adv 25:294–306
- Chu WL, Phang SM, Goh SH (1996) Environmental effects on growth and biochemical composition of *Nitzschia inconspicua*. Grunow. J Appl Phycol 8:389–396
- Coombs J, Spanis C, Volcani BE (1967) Studies on the biochemistry and fine structure of silica shell formation in diatoms. Photosynthesis and respiration in silicon-starvation synchrony of *Navicula pelliculosa*. Plant Physiol 42:1607–1611
- Fields M, Hise A, Lohman E, Bell T, Gardner R, Corredor L, Moll K, Peyton B, Characklis G, Gerlach R (2014) Sources and resources: Importance of nutrients, resource allocation, and ecology in microalgal cultivation for lipid accumulation. Appl Microbiol Biotechnol 98:4805–4816
- Griffiths MJ, Hille RP, Harrison STL (2012) Lipid productivity, settling potential and fatty acid profile of 11 microalgal species grown under nitrogen replete and limited conditions. J Appl Phycol 24:989–1001
- Huerlimann R, de Nys R, Heimann K (2010) Growth, lipid content, productivity, and fatty acid composition of tropical microalgae for scale-up production. Biotechnol Bioeng 107:245–257
- Jiang Y, Yoshida T, Quigg A (2012) Photosynthetic performance, lipid production and biomass composition in response to nitrogen limitation in marine microalgae. Plant Physiol Biochem 54:70–77
- Jiang Y, Lavery KS, Brown J, Nunez M, Brown L, Chagoya J, Burow M, Quigg A (2014) Effects of fluctuating temperature and silicate supply on the growth, biochemical composition and lipid accumulation of *Nitzschia* sp. Bioresour Technol 154:336–344
- Kyle DJ, Gladue RM (1991) Eicosapentaenoic acids and methods for their production. PCT Patent Application WO 91/14427
- Nalewajko C (1966) Dry weight ash and volume data for some freshwater planktonic algae. J Fish Res Board Can 23:1285–1288
- Parslow JS, Harrison PJ, Thompson PA (1984) Saturated uptake kinetics: transient response of the marine diatom *Thalassiosira pseudonana* to ammonium, nitrate, silicate or phosphate starvation. Mar Biol 83: 51
- Revellame E, Hernandez R, French W, Holmes W, Alley E (2010) Biodiesel from activated sludge through in situ transesterification. J Chem Tech Biotechnol 85:614–620
- Roessler PG (1988a) Changes in the activities of various lipid and carbohydrate biosynthetic enzymes in the diatom *Cyclotella cryptica* in response to silicon deficiency. Arch Biochem Biophys 267:521–528
- Roessler PG (1988b) Effects of silicon deficiency on lipid composition and metabolism in the diatom *Cyclotella cryptica*. J Phycol 24:394–400
- Sheehan J, Dunahay T, Benemann J, Roessler P (1998) A look back at the U.S. Department of Energy's Aquatic Species Program—biodiesel from algae. National Renewable Energy Laboratory, Golden, pp 1–328, NREL/TP-580-24190
- Spolaore P, Joannis-Cassan C, Duran E, Isambert A (2006) Commercial applications of microalgae. J Biosci Bioeng 101:87–96

- Taguchi S, Hirata JA, Laws EA (1987) Silicate deficiency and lipid synthesis of marine diatoms. *J Phycol* 23:260
- Thamatrakoln K, Hildebrand M (2008) Silicon uptake in diatoms revisited: a model for saturable and nonsaturable uptake kinetics and the role of silicon transporters. *Plant Physiol* 146:1397–1407
- Tyrrell T, Law CS (1997) Low nitrate-phosphate ratios in the global ocean. *Nature* 387:793–796
- UNE-EN 14214 (2003) Automotive fuels, fatty acid methyl esters (FAME) for diesel engines, requirements and test methods
- Wen ZY, Chen F (2000) Heterotrophic production of eicosapentaenoic acid by the diatom *Nitzschia laevis*: effects of silicate and glucose. *J Ind Microbiol Biotechnol* 25:218–224
- Yu ET, Zendejas FJ, Lane PD, Gaucher S, Simmons BA, Lane TW (2009) Triacylglycerol accumulation and profiling in the model diatoms *Thalassiosira pseudonana* and *Phaeodactylum tricorutum* (Bacillariophyceae) during starvation. *J Appl Phycol* 21:669–681
- Zhu C, Lee Y (1997) Determination of biomass dry weight of marine microalgae. *J Appl Phycol* 9:189–194