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Effects of fluctuating temperature and silicate supply on the growth, biochemical composition and lipid accumulation of *Nitzschia* sp.

Yuelu Jiang [a,*], Katherine Starks Laverty [a], Jola Brown [b], Marcella Nunez [a], Lou Brown [b], Jennifer Chagoya [c], Mark Burow [c], Antonietta Quigg [a,d]

[a] Department of Marine Biology, Texas A&M University at Galveston, Galveston, 77553 TX, USA
[b] Texas Agrilife Research, Texas A&M University, Pecos, 79772 TX, USA
[c] Texas AgriLife Research, Texas A&M System, Lubbock, 79403 TX, USA
[d] Department of Oceanography, Texas A&M University, College Station, 77843 TX, USA

HIGHLIGHTS

- Low Si limited growth in both growth and lipid formation media under 3 seasons.
- Winter condition introduced higher percentage of unsaturated fatty acids.
- Saturated fatty acids and monounsaturated fatty acids were most abundant in summer.
- In winter, poly-unsaturated fatty acids increased with the increase of Si.

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ABSTRACT

Nitzschia sp. (Bacillariophyceae) was grown under temperature and photoperiods mimicking those, typical during summer, spring/fall and winter conditions in the southern United States, and using five silicate (Si) concentrations. In general, higher Si concentrations resulted in higher growth rates in summer and spring/fall conditions and lower organic content. Si-deficient *Nitzschia* sp. had higher levels of neutral lipid compared to those growing in Si replete media. Under summer conditions, the proportion of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) was relatively stable compared with spring/fall and winter conditions, and the proportion of polyunsaturated fatty acids (PUFA) was low. In the winter condition, SFA and MUFA showed a gradient of decreasing abundance while PUFA gradients increased with increasing Si concentrations in the medium. Cumulative productivity (optimization of growth and lipid content) would be best in the spring/fall but less so in the other conditions for this strain of *Nitzschia* sp.

1. Introduction

Microalgae efficiently convert CO_2 to potential biofuels, feeds and high-value byproducts using a small foot print (Chisti, 2007). Microalgae can grow on non-arable land and use non-potable water without displacing food crops. This growth is considered environmental friendly as microalgal biofuels can take advantage of nutrients in wastewater and CO_2 from power plants; while crop plants cannot use these resources (Chisti, 2007). Other factors, which should be considered simultaneously for sustainable biofuel production include but are not limited to: lipid and high added-value chemicals production (e.g. for pharmaceutical or cosmetic industry), extraction economics (solvents, ultrasound application, electromagnetic field use, etc.), incineration/pyrolysis/gasification of residual biomass, its anaerobic digestion for biogas production, etc. These factors have been reviewed recently in Chisti (2007), Schenk et al. (2008) and Šoštarič et al. (2012).

Issues in large scale biofuels production with microalgae in open ponds are contamination, lack of control over temperature and light, loss of water by evaporation, grazing by protozoa and zooplankton, fungal parasites and viral infections. Pulz and Gross (2004) reported that successful algal biotechnology mainly depends on choosing the right alga with relevant properties for specific culture conditions and products. Large-scale production

Abbreviations: AFDW, ash-free dry weight; C, carbon; DW, dry weight; FAMEs, fatty acid methyl esters; GC, gas chromatography; MS, mass spectrometer; MUFA, mono-unsaturated fatty acids; NR, Nile Red; N, nitrogen; OD, optical density; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; Si, silicate.

* Corresponding author. Present address: Institute of Ocean Science and Technology, Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, China. Tel.: +86 755 2603 6257; fax: +86 755 2603 6322.
E-mail address: jiang.yuelu@sz.tsinghua.edu.cn (Y. Jiang).

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of microalgal biomass therefore requires species, which also can potentially be tolerant of a wide range of conditions. Two strategies are generally considered: (1) use a single species year round or (2) use several different species, alternating them between seasons. Both approaches are driven by the need to optimize growth and lipid production. Each has advantages and disadvantages. Using one species year round may reduce the potential for contamination with other microalgae – the tradeoff is that at different times of the year, growth and lipid production may not be as high. Using a specific species for each season requires additional handling procedures. Griffiths and Harrison (2009) summarize these and other considerations. Further, microalgae in nature will experience environmental fluctuations; this maybe due to the variable supply of sunlight throughout the day and varying cloud cover, temperatures in the field are variable not only daily but on seasonal cycles. Constant light and temperature treatments used in the laboratory hence cannot reflect ecologically realistic environments.

The production and storage of lipid by microalgae is also regulated by nutrients, especially nitrogen (N), phosphorus (P) and silicate (Si). Diatoms are one of the largest groups of silicifying organisms and most species have an obligate requirement for Si for cell wall formation. This characteristic means that in addition to modifying nutrients such as N and P in the media to induce lipid formation (Griffiths and Harrison, 2009; Jiang et al., 2012; Kwon et al., 2013), the Si concentration in manipulated to alter growth rates and the macromolecular composition of diatom cells. Previous studies have shown that lipids accumulate very slowly during either N as nitrate or P deficiency compared to Si deficiency (Shifrin and Chisholm, 1981; Werner, 1977), which is especially true in the case of diatoms. Werner (1977) reported that the production of fatty acids in diatoms increased by more than 100% for 6–9 h following the transfer from a media with Si to a medium without Si. Also Shifrin and Chisholm (1981) reported that the production of lipid material per diatom cell doubled within 12 h following the onset of Si deficiency.

In the current study, the diatom *Nitzschia* sp. (Bacillariophyceae) was grown under three laboratory cycles designed to stimulate seasonal conditions (summer, spring/fall and winter) to examine changes in its growth, protein, lipid content and fatty acid profile. This study also examined the response of *Nitzschia* sp. to five different Si concentrations (treatments) through the growth phase (GP), and then by omitting Si from the media, during the lipid formation phase (LP). In this study, *Nitzschia* sp. varies growth, biomass and lipid production in a complex manner in response to the changes in season (conditions) and Si in the media (treatments). These results reveal that *Nitzschia* sp. produced the most lipids during winter conditions and when grown in media not supplemented with Si; but this was also when growth was the slowest.

Table 1
Temperature ramping and light:dark cycles used to grow *Nitzschia* sp. under conditions reflecting typical summer, spring/fall and winter in Midland, Texas.

Time	Temperature (°C)			Light		
	Summer	Spring/fall	Winter	Summer	Spring/fall	Winter
8:00 AM	25	13	4	On	On	On
10:00 AM	30	17	8	On	On	On
12:00 PM	32	21	13	On	On	On
2:00 PM	34	25	17	On	On	On
4:00 PM	37	23	16	On	On	On
6:00 PM	35	21	14	On	On	Off
8:00 PM	33	19	10	Off	Off	Off
10:00 PM	30	17	7	Off	Off	Off
12:00 AM	28	15	5	Off	Off	Off
2:00 AM	26	13	4	Off	Off	Off
4:00 AM	26	11	3	Off	Off	Off
6:00 AM	23	10	2	On	Off	Off

2. Methods

2.1. Isolation

A native diatom strain, *Nitzschia* sp. (TAMU-LBK-017) was isolated from a seasonal playa lake in Lubbock, Texas (USA) by streaking water samples onto a series of agar plates prepared with artificial growth phase media (see below). 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 determine the species identity. Native strains were chosen in anticipation of a more successful transition to large-scale production.

2.2. Cultivation conditions

Nitzschia sp. was grown under cycles chosen to mimic average diurnal fluctuating temperature and photoperiods (day length) typically experienced during the major seasonal periods: summer, spring/fall and winter. These were designed using the average temperature and photoperiods recorded in Midland, Texas (Table 1) during 2007–2010 at the local meteorological station (Texas High Plains Evapotranspiration Network).

All diatom experiments were conducted in a Caron diurnal incubator (6020 series) which allowed the temperature ramping and the photoperiod cycles used in this study (Table 1). The light intensity ranged between 130 and 150 μmol photons m^{-2} s^{-1}. All cultures were grown in triplicate acid-washed 1 L polycarbonate bottles, stirred with IKA stir plate (IKAMAGRO 15 power) on the rate of 180 rpm, in order to ensure continuous mixing throughout the growth and lipid formation phases.

An artificial brackish growth media was developed using the groundwater from Midland, Texas. The artificial brackish growth media developed for this study included monoammonium phosphate ($NH_4H_2PO_4$, 0.26 mol m^{-3}) as the P source and urea (CH_4N_2O, 4.26 mol m^{-3}) as N source. For Si treatments, final concentrations of Si were 0.2, 1.1, 2.1, 4.2 and 10.6 mol m^{-3}. Experimental growth medium contained 1 mL L^{-1} of Gaffron's trace metal solution and 1 mL L^{-1} f/2 vitamins. The medium was adjusted to 15 PSU salinity with NaCl (Tru-soft, solar crystals, United Salt Corp.). $NaHCO_3$ was added (saturated solution) as the carbon source to avoid carbon limitation at high cell densities and to help stabilize the pH to around 8–8.2.

Nitzschia sp. was grown for three–five generations in order to acclimate to the three seasonal conditions and five Si concentrations (treatments) ranging from 0.21 to 10.6 mol m^{-3} Si. Once the cultures had acclimated, the growth rate was measured. Measurements were performed during the exponential phase; these findings will be 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 media was replaced with fresh media lacking Si but with the same N and P concentrations. Cultures were monitored as during the GP. For simplicity, LP treatments will be referred by the corresponding GP Si concentrations. The duration of LP incubation varied for each season (condition), 5 days for summer, 4 days for spring/fall and 6–11 days for winter.

2.3. Growth rates

Changes in cell density were followed by measuring OD_{750nm} using a spectrophotometer (Shimadzu UV/VIS-2501) with fresh