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Artículo Original

Growth rate, lipid, fatty acids, and pigments content of *Melosira moniliformis* (Bacillariophyta) in laboratory cultures

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Abstract

The diatom *Melosira moniliformis* was isolated from the intertidal zone of *Laguna de La Paz* in the State of Baja California Sur in Mexico. The biotechnological potential of this strain for lipid and fatty acid production was evaluated in laboratory cultures. The strain was grown at 21 °C with moderate agitation and 26 °C without agitation, with moderate agitation and vigorous agitation. Growth and biomass, pigments, lipids, and fatty acid production were measured. At 21 °C growth showed a longer lag stage but there was no difference in growth rate (0.68–0.72 d^{-1}) or biomass production (0.44–0.60 g L^{-1}) between the culture conditions of temperature or agitation. Lipid content varied from 17.38–26.65% of organic biomass, without difference between culture conditions. The highest fatty acids content observed were C14:0, C16:0, C16:1n7, and eicosapentaenoic acid (EPA) C20:5n3; this last fatty acid reached up 50% (50.4–51.6 mg g^{-1} organic dry weight) but without differences between culture conditions. *M. moniliformis* can be cultivated under planktonic (vigorous agitation) or benthic conditions (without agitation) at 26 °C. Its morphological characteristics and the tendency to form chains could be an advantage for lower harvesting costs, and the high EPA content, indicate a high potential for use in biotechnology.

Key words*: Biovolume; Coscinodiscophyceae; diatoms culture; PUFA*

Resumen

La diatomea *Melosira moniliformis* fue aislada de la zona intermareal de la Laguna de La Paz en el estado de Baja California Sur en México. El potencial biotecnológico de esta cepa fue evaluado en cultivos de laboratorio para la producción de lípidos y ácidos grasos. La cepa se cultivó a 21 °C con agitación moderada y a 26 °C sin agitación, con agitación moderada y agitación vigorosa. Se midió el crecimiento y la biomasa, la producción de pigmentos, lípidos y ácidos grasos. A 21 °C el crecimiento mostró una fase lag más larga, pero no hubo diferencia en la tasa de crecimiento (0.68-0.72 d^{-1}) o la producción de biomasa (0.44-0.60 g L^{-1}) entre las condiciones de cultivo de temperatura o agitación. El

contenido de lípidos varió de 17.38-26.65% de la biomasa orgánica, sin diferencia entre las condiciones de cultivo. Los contenidos de ácidos grasos más altos observados fueron C14:0, C16:0, C16:1n7 y ácido eicosapentaenoico (EPA) C20:5n3; este último ácido graso alcanzó hasta 50% (50.4-51.6 mg g^{-1} del peso seco orgánico) pero sin diferencias entre las condiciones de cultivo. *M. moniliformis* se puede cultivar bajo condiciones planctónicas (agitación vigorosa) o bentónicas (sin agitación) ac26 °C. Sus características morfológicas y la tendencia a formar cadenas podrían ser una ventaja para reducir costos de cosecha, y el alto contenido de EPA indica un alto potencial para su uso en biotecnología.

Palabras clave: *Biovolumen; Coscinodiscophyceae; cultivo de diatomeas; PUFA.*

1. Introduction

Diatoms are an abundant and diverse group of microalgae that live in terrestrial and marine environments; they are responsible of the 20–25% of net primary global productivity (Nelson *et al.*, 1995; Falkowsky and Raven, 2007), and they contribute 40% of primary annual marine productivity (Field *et al.*, 1998; Thornton, 2012). In spite of these qualities, there are few species that have been used for biotechnological purposes, due in part to the scarce information on their reproductive biology (Davidovich *et al.*, 2015) and that products of interest are not well characterized (Lincoln *et al.*, 1990; Moore, 1999; Lebeau and Robert, 2003). One of the main uses of diatoms has been in aquaculture as live food for invertebrates and zooplankton for fish larvae (Brown, 2002; Guedes and Malcata, 2012). Genera used in aquaculture include *Skeletonema*, *Chaetoceros*, *Phaeodactylum*, *Odontella*, *Thalassiosira*, *Nitzschia*, and *Amphora* (Talebi *et al.*, 2013; Xia *et al.*, 2013; Mata *et al.*, 2010; Song *et al.*, 2013), some of them are generally considered as safe (Gangl *et al.*, 2015). They are notable for high nutritive value, especially fatty acids and essential amino acids (Lombardi and Wangersky, 1995; Muller-Feuga *et al.*, 2003; Guedes and Malcata, 2012). Some species of diatoms have high lipid production; these have been considered promising organisms for production of biofuels and application in nanotechnology, pharmacology, cosmetology, and bioremediation of wastewater (Lebeau and Robert, 2003; Bozarth *et al.*, 2009; Hildebrand *et al.*, 2012; Odjadjare *et al.*, 2015; Wang and Seibert, 2017).

Currently, some of the most common genera with potential applications in biotechnology are: *Amphora*, *Odontella*, *Thalassiosira*, *Skeletonema*, and *Phaeodactylum* (Mata *et al.*, 2010; Song *et al.*, 2013; Talebi *et al.*, 2013; Xia *et al.*, 2013).

The genus *Melosira* includes freshwater species, e.g., *M. varians* (Wendel and Jüttner, 1996; Piloni *et al*. 2017) and marine species, e.g., *M. nummuloides* (McLean *et al.*, 1981; Yamazaki *et al.*, 2010; Woelfel *et al.*, 2014). Currently, 71 taxa belong to this genus in terrestrial, mixohaline, and marine environments (Guiry and Guiry, 2017). *M. varians* and *M. nummuloides* have been the topic of study for a few decades (McLean *et al.*, 1981; Wendel and Jüttner, 1996; Yamazaki *et al.*, 2010), but *M. moniliformis* has been less studied (Woelfel *et al.*, 2014).

Assessment of the biotechnological potential of a species begins with measuring growth and production of

organic products under controlled laboratory conditions. The biochemical composition these microorganisms can be influenced by altering the proportions of nutrients, e.g. the decrease of silicates and nitrates in general produce changes in the percentage and diversity of lipids, principally related to increases in saturated and decreases of polyunsaturated fatty acids (PUFAs) (Chauton *et al.*, 2013; Mooij *et al.*, 2015), what has been used to propose diatoms as candidates to biofuels production.

In this work, we report the growth, lipid and fatty acid production, pigment production (chlorophyll *a*, *c* and total carotenoids) under two different temperatures and three agitation conditions (moderate, vigorous, and none), all of this to evaluate the biotechnological potential of *M. moniliformis*.

2. Materials and methods

2.1. *Collection and management of strain*

Melosira moniliformis was collected in intertidal zone of *Laguna de La Paz*, B.C.S., Mexico (24°8′13.98″N, 110°25′31.21″W). It was isolated as a single-cell by micropipette and in agar as a monoclonal culture and maintained in f/2 media (Guillard, 1975) at salinity of 35 UPS. Taxonomic identification was done by observation of cell walls by electron microscopy, following the technique reported by López-Fuerte *et al*. (2016) and employing a scanning electron microscope Jeol JSM–7600F. Taxonomy was possible in accord to Crawford (1977) (Fig. 1).

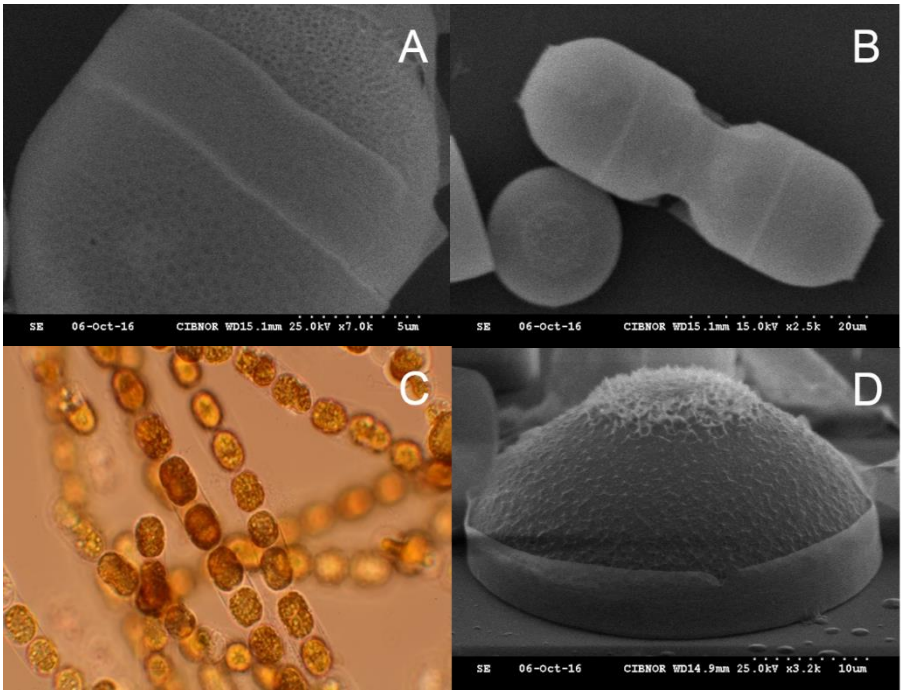

Figure 1. Different images of *Melosira moniliformis* obtained by light and electron microscopy. Silica frustule including valves and girdle bands in pleural view **(A)**, diatom during cell division **(B)**, cells arranged in chains **(C)** little rimoportulae, areolas and central rosette **(D)**.

The experiments were performed by triplicate in 1 L flasks with 500 mL medium. The inoculum was added at 10 % of volume at exponential phase, with filtered aeration at 1 µm (0.9-1 L min^{-1}), photoperiod 12:12 under white fluorescent side lighting at irradiance of 70 µmol photon $m^{-2}·s^{-1}$. Growth was measured during four days; the cultures were maintained at 21 ± 1 °C and 26 ± 1°C. Cultures at 21 ± 1 °C and 26 ± 1°C were under same inoculum culture parameters with manual moderate agitation, and those at 26 ± 1°C were also maintained in other two agitation conditions, manual vigorous agitation twice a day to simulate planktonic culture growth and no agitation for benthic growth. Every day at the same time (09:00 h), samples of all treatments except those without agitation, were taken to determine optical density and cell density. Samples were processed for counts according to Richmond and Hu (2013). The content of pigments (chlorophyll *a*, *c*, and total carotenoids) was determined every day to the moderate and vigorous agitated cultures. At the end of the experiment, biomass was harvested by decantation and centrifugation, freeze-dried, and maintained at -20 °C, until lipid and fatty acid content was analyzed few days after.

2.2. *Cellular features*

Cells of *M. moniliformis* obtained from the initial inoculum in exponential stage were measured (diameter and pervalvar axis) and biovolume was calculated, assuming a cylindrical body shape, according to Hillebrand (1999) and Olenina *et al.* (2006). The dry cell weight, percentage of ash dry weight, and ash free dry weight was determined, according to Richmond and Hu (2013) (Table 1).

Table 1. Measurements of size and weight of *Melosira moniliformis* cells (mean ± SEM). (n = 30).

Cell measurements	
Diameter (µm)	20.97 ± 0.29
Pervalvar axis (µm)	24.85 ± 0.17
Biovolume ($µm^3$)	8666 ± 291
Dry weight (mg × 10^6 cells)	9.69 ± 0.05
Ash weight (%)	67–76
Ash free dry weight (%)	24–33

2.3. *Cell growth*

The cell density was calculated, using a correlation curve optical density vs cellular density at 680 nm. Cell counts were performed in a Neubauer chamber. Growth rate (μ) was determined by the expression: $\mu = (\ln Df - \ln Di) / (tf - ti)$, where *D*i and *D*f are the cellular densities at initial and final time (*t*i and *t*f) (Guillard *et al.*, 1973). Growth curves were obtained for all treatments, except for the cultures at 26 °C without agitation.

2.4. *Pigments*

A sample of 2 mL of each culture was centrifuged at 10 000 rpm at 10 °C for 4 min and the pellet was added with 2 mL of 90% acetone for determination of pigments. Chlorophyll *a* was determined, according to Jeffrey and Humphrey (1975) and chlorophyll *c* and

total carotenoids were determined in accord to Parsons *et al.* (1984).

2.5. *Lipids and fatty acids*

The biomass was lyophilized to be used in different analyzes, the lipids were determined according to the method of Folch *et al.*, (1957). Fatty acids were quantified in extracted lipids, which were trans-esterified directly with methanol acid at 90 °C for 90 min and extracted with heptane and water. The heptane fraction was analyzed by FID gas chromatography (Agilent 7820A), with a capillary column (J&W DB 23 122-2332, 250 °C, 30 m, 0.25 mm, 0.25 μm), using helium as a carrier gas, the method was: 50 °C for one minute, gradient of 25 °C min^{-1} to reach 175 °C for 3 min, and gradient of 1.9 °C min^{-1} to reach 220 °C for 10 min, followed by a 230 °C post run for 10 min. Chromatograms were interpreted by comparison with the retention times and correction factors of a FAME blend standard (CRM47885**,** Supelco). In cases where some fatty acids could not be identified with the standard, they were determined by gas mass chromatography (G1800B, Agilent Technologies).

2.6. *Statistical analysis*

Parametric data were analyzed by ANOVA; percentage, rate, or concentration data were transformed (Zar, 2005) before analyzing by ANOVA. When there were significant differences, the Tukey test was applied. Level of significance was set at $p < 0.05$. Analysis were carried out with STATISTICA 10.0 software.

3. Results

3.1. *Cell features*

The fig. 1C shows the tendency of *M. moniliformis* to form chains in all cultures and the ultrastructure showing the very dense cell wall, there are little rimoportulae (Fig. 1D), probably related with secretion of mucous substances that maintain connection between the cells that form chains. This structure is probably responsible for the higher than 50 % ash content observed. The cell wall measurements, biovolume, and cellular weight of *M. moniliformis* that are presented in Table 1 correspond to a sample of cells used in the inoculum, which culture condition were at 26 ° C with moderate agitation.

3.2. *Growth*

Growth curves showed the effect of temperature (Fig. 2), with a major latency at 21 °C than 26 °C, which was manifested in minor growth in the first three days in this condition. At day 3 of culture, the growth was major at 26 °C with the condition of vigorous agitation. In all cases, it was observed exponential stage, but only at 26 °C with vigorous agitation there was a clear rise to the plateau stage after day 3. At the end of culture, we did not observe a significant difference between cell densities with different treatments, but it was observed a tendency for a higher growth at higher temperature with vigorous agitation (Table 2). At the end of the experiment, the dry and ash free dry weights were between 418–596 mg L^{-1} and 0.114–0.154 mg L^{-1}, respectively, without difference between treatments. Growth rates did not showed difference, with mean values of 0.72, what indicates a mean duplication time of 1.03 d^{-1}. There was no difference in productivity expressed as dry weight, ash free dry weight, or final cell density.

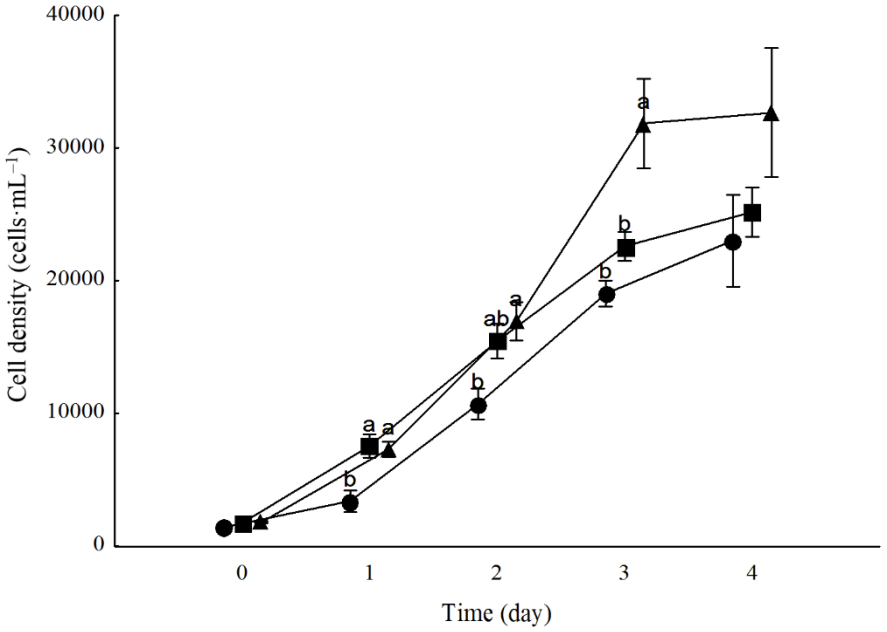

Figure 2. Mean cell density ± SE of *Melosira moniliformis*, cultivated at 21 ± 1 °C moderate agitation (circles), 26 ± 1°C moderate agitation (squares), and 26 ± 1 °C vigorous agitation (triangles). Different letters indicate significant differences (n = 3, ANOVA, Tukey test, $p < 0.05$, b < a).

Table 2. Growth rate (μ), dry and ash free dry weight productivity (P_{DW}, P_{AFDW}) and final cell density (mean ± SEM). (n = 3, $p > 0.05$). There was no significant difference.

	21 °C [1]	**26 °C [1]**	**26 °C [2]**	**26 °C [3]**
$\boldsymbol{\mu}$ (d^{-1})	0.71 ± 0.04	0.68 ± 0.04	0.77 ± 0.03	0.72 ± 0.03
$\mathbf{P_{DW}}$ (g L^{-1} d^{-1})	0.14 ± 0.02	0.11 ± 0.01	0.15 ± 0.01	0.15 ± 0.03
$\mathbf{P_{AFDW}}$ (g L^{-1} d^{-1})	0.03 ± 0.01	0.03 ± 0.00	0.05 ± 0.01	0.04 ± 0.01
Final cell density (10^4 cells m^{-1})	2.30 ± 0.43	2.52 ± 0.23	3.23 ± 0.30	3.27 ± 0.60

[1]Moderate agitation; [2]without agitation; [3]vigorous agitation.

3.3. *Pigments*

Pigment content, as a function of organic biomass (Fig. 3), increased in chlorophyll *a*, *c*, and total carotenoids as the culture grew, without differences between treatments. On day 3, there was a difference in total carotenoid content, with higher values at 21 °C. Production of pigments, as a function of organic biomass (Fig. 4), was significantly in chlorophyll *c*, with the lowest values at 26 °C without agitation. For productivity, significant differences were present in chlorophyll *c* and total carotenoids, with lowest values at 26 °C without agitation and at 21 °C.

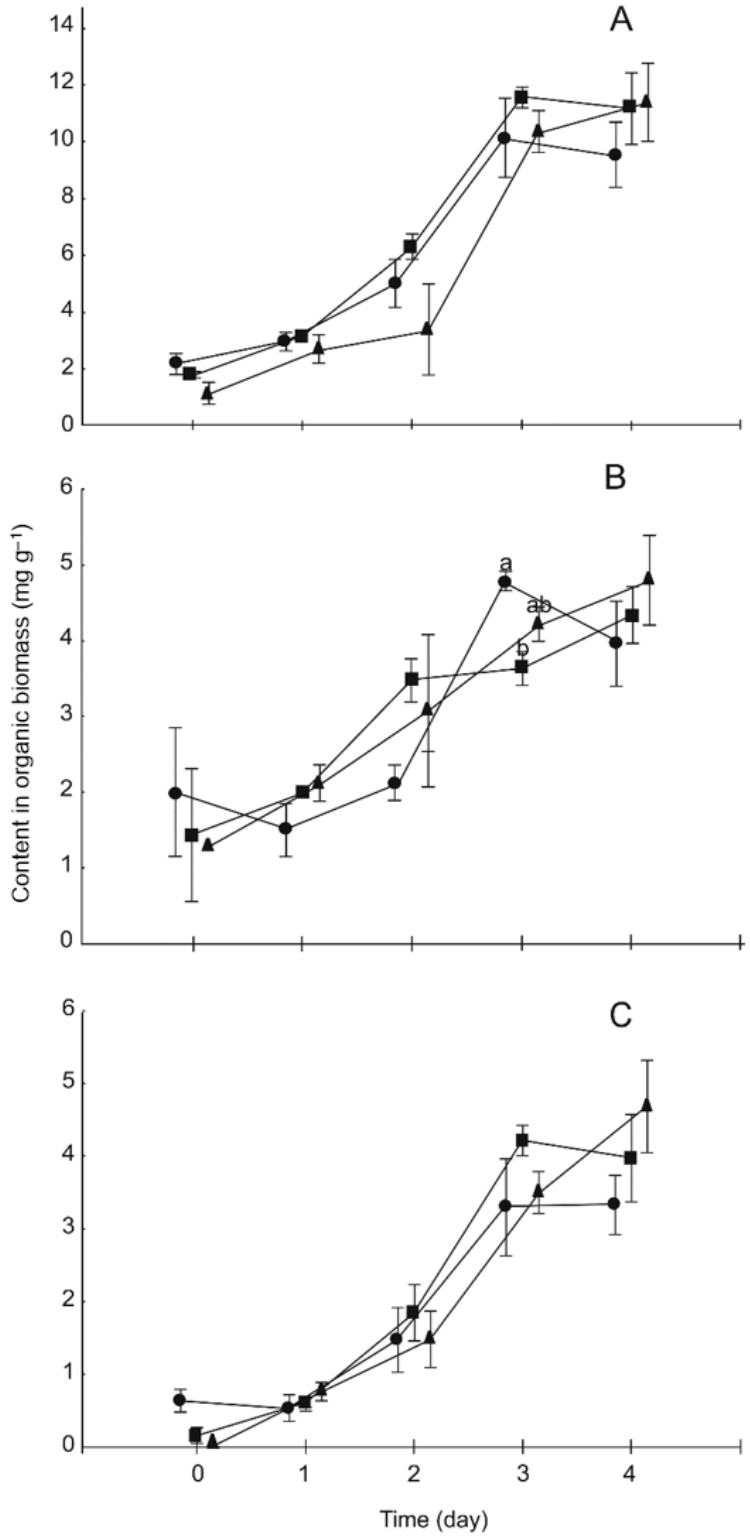

Figure 3. Chlorophyll a **(A)**, chlorophyll c **(B)**, and total carotenoids **(C)** content in organic biomass, mean ± SE of *Melosira moniliformis*, cultivated at 21 ± 1 °C moderate agitation (circles), 26 ± 1 °C moderate agitation (squares), and 26 ± 1 °C vigorous agitation (triangles). Different letters indicate significant differences (n = 3, $p < 0.05$, b < a).

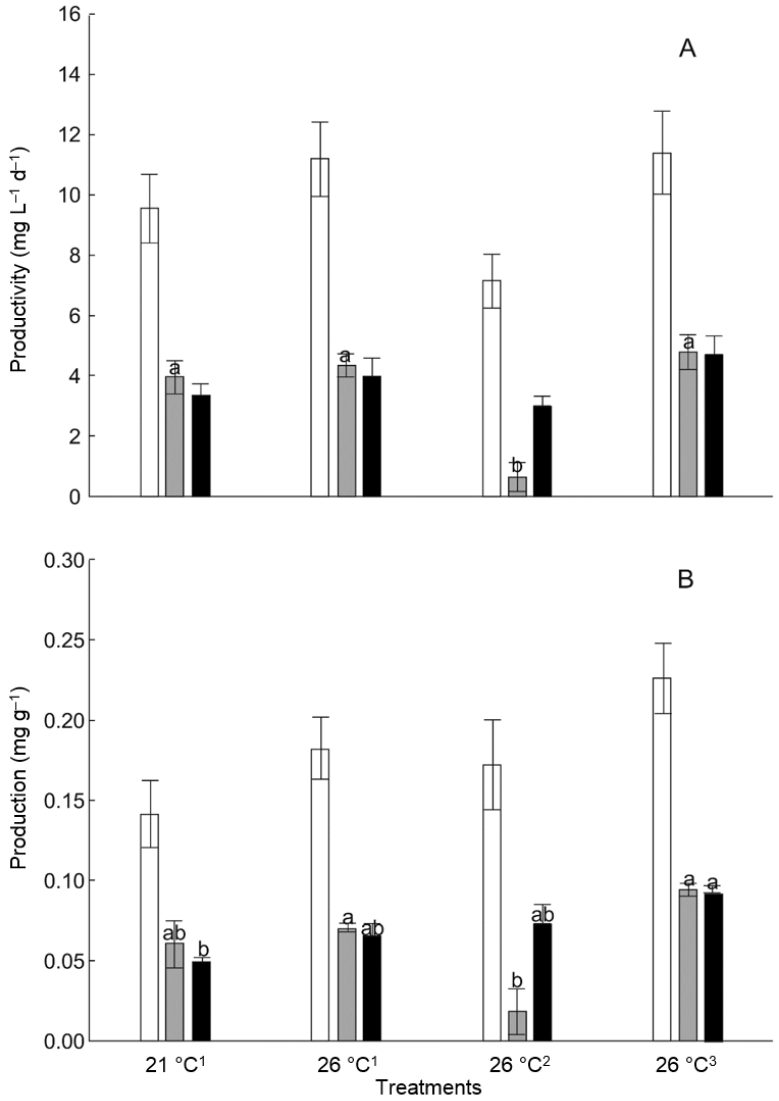

Figure 4. Pigments production **(A)** and productivity **(B)**, mean ± SE of *Melosira moniliformis* organic biomass at the end of the experiment, cultivated at 21 ± 1 °C moderate agitation (21 °C[1]), 26 ± 1 °C moderate agitation1 (26 °C[1]), 26 ± 1 °C without agitation (26 °C[2]), and 26 ± 1 °C vigorous agitation (26 °C[3]). Chlorophyll *a* (light bars), chlorophyll *c* (gray bars), and total carotenoids (dark bars). Different letters indicate significant differences between means. (n = 3, $p < 0.05$, b < a).

3.4. *Lipids and fatty acids*

The highest lipid content on a dry basis occurred at 26 °C without agitation, which was almost double that found at 21 °C and 26 °C with moderate and vigorous agitation respectively (Table 3). The lipid content respect to ash free dry weight showed similar results, with percentages between 17–26%, what reflex the characteristic of having a high percentage of inorganic matter (25–30%) forming part of the cell wall (Tables 1 & 2).

Table 3. Total lipid content at the end of the experiment in percentages of dry (% DW) and ash free dry weight (% AFDW) of *Melosira moniliformis* at the end of the experiment (mean ± SE).

Treatments	% DW	% AFDW
21 °C [1]	4.81 ± 0.52 b	17.82 ± 1.14 b
26 °C [1]	6.67 ± 0.22 ab	24.65 ± 1.50 ab
26 °C [2]	8.30 ± 1.15 a	26.10 ± 4.38 a
26 °C [3]	4.44 ± 0.46 b	17.38 ± 3.13 b

[1]Moderate agitation; [2]without agitation; [3]vigorous agitation.
Different letters indicate significant difference (n = 3, $p < 0.05$, b < a).

Among fatty acids (Table 4), saturated fatty acids (SFAs) represented the highest fraction (45.59%); of these, the main were 14:0 and 16:0 with 22.20% and 73.50%. Monounsaturated fatty acids (MUFAs) and PUFAs were present at 25.27% and 29.13%, with no difference between treatments. The major monosaturated fatty acid was C16:1n7 at 91.09%. The major PUFAs were eicosapentaenoic acid (EPA) C20:5n3, C16:3n4, C16:2n3, C18:4n3, and docosahexaenoic acid (DHA) C22:6n3, with percentages of 51.12, 16.34, 8.81, 6.29, and 6.19%, respectively. Fatty acid content related to organic biomass only showed significant difference in the case of arachidonic acid (ARA) C20:4n6, with content of <0.5%, and greater content under 26 °C with moderate agitation and 26 °C without agitation. The ARA/DHA ratio was lower for 21 °C without differences with the other treatments. No significant differences were present in DHA/EPA ratios.

Table 4. Fatty acids content of *Melosira moniliformis* organic biomass ($mg \cdot g^{-1}$) mean values ± SEM. Saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) (mean ± SEM) as % of total fatty acids.

Fatty acid	21 °C[1]	26 °C[1]	26 °C[2]	26 °C[3]
12:0	0.10 ± 0.01	0.17 ± 0.05	0.13 ± 0.06	0.07 ± 0.02
14:0	8.10 ± 0.71	13.29 ± 1.93	15.96 ± 7.72	8.10 ± 2.52
i14:0	0.18 ± 0.08	0.29 ± 0.07	0.16 ± 0.06	0.15 ± 0.02
15:0	0.34 ± 0.14	0.60 ± 0.08	0.70 ± 0.30	0.40 ± 0.11
16:0	28.75 ± 2.75	47.70 ± 7.86	64.74 ± 29.80	28.16 ± 8.40
18:0	0.71 ± 0.07	1.59 ± 0.69	3.09 ± 1.80	0.74 ± 0.16
20:0	0.05 ± 0.01	0.09 ± 0.03	0.11 ± 0.05	0.05 ± 0.01
22:0	0.11 ± 0.03	0.18 ± 0.05	0.18 ± 0.06	0.09 ± 0.02
24:0	0.06 ± 0.01	0.10 ± 0.01	0.10 ± 0.03	0.05 ± 0.01
16:1n7	19.97 ± 2.03	32.17 ± 4.83	42.82 ± 19.73	19.53 ± 5.75
16:1n5	0.31 ± 0.03	0.49 ± 0.11	0.48 ± 0.20	0.32 ± 0.07
16:1n3	0.16 ± 0.02	0.22 ± 0.03	0.21 ± 0.07	0.17 ± 0.03
18:1n11	0.15 ± 0.02	0.20 ± 0.02	0.18 ± 0.05	0.16 ± 0.04
18:1n9	0.45 ± 0.03	0.93 ± 0.21	1.54 ± 0.75	0.54 ± 0.16
18:1n7	0.95 ± 0.42	1.00 ± 0.25	0.80 ± 0.22	0.61 ± 0.09
20:1n9	0.03 ± 0.00	0.07 ± 0.02	0.05 ± 0.01	0.04 ± 0.00
22:1n9	0.10 ± 0.02	0.11 ± 0.03	0.11 ± 0.04	0.07 ± 0.02
24:1n9	0.43 ± 0.06	0.47 ± 0.07	0.43 ± 0.13	0.34 ± 0.06
16:2n6	0.75 ± 0.13	0.95 ± 0.09	0.68 ± 0.18	0.75 ± 0.13
16:2n3	2.92 ± 0.58	3.68 ± 0.34	2.80 ± 0.71	2.99 ± 0.52
16:3n6	0.07 ± 0.02	0.13 ± 0.06	0.19 ± 0.08	0.08 ± 0.01
16:3n4	5.66 ± 1.35	7.18 ± 0.65	5.20 ± 1.28	5.09 ± 0.58
17:2	0.10 ± 0.01	0.09 ± 0.01	0.08 ± 0.02	0.08 ± 0.01
16:4	0.05 ± 0.01	0.05 ± 0.02	0.07 ± 0.03	0.06 ± 0.00
18:2n6	0.54 ± 0.05	1.04 ± 0.18	1.33 ± 0.54	0.67 ± 0.16
18:3n6	0.32 ± 0.04	0.71 ± 0.12	0.87 ± 0.35	0.44 ± 0.10
18:3n3	0.04 ± 0.01	0.06 ± 0.01	0.06 ± 0.03	0.05 ± 0.01
18:4n?	0.39 ± 0.36	0.12 ± 0.08	0.25 ± 0.05	0.06 ± 0.01
18:4n3	2.03 ± 0.30	2.56 ± 0.46	3.64 ± 1.64	1.36 ± 0.33
20:3n6	0.12 ± 0.02	0.29 ± 0.04	0.33 ± 0.11	0.20 ± 0.06
$20:4n6_{ARA}$	0.19 ± 0.08 b	1.03 ± 0.13 a	0.94 ± 0.24 a	0.68 ± 0.17 ab[*]
20:3n3	0.54 ± 0.09	0.61 ± 0.10	0.76 ± 0.30	0.37 ± 0.08
$20:5n3_{EPA}$	16.94 ± 3.60	22.26 ± 3.05	20.75 ± 6.42	14.27 ± 2.29
$22:6n3_{DHA}$	2.10 ± 0.47	2.69 ± 0.39	2.50 ± 0.77	1.71 ± 0.33
ΣSFA	38.39 ± 3.54	64.01 ± 10.74	85.15 ± 39.53	37.81 ±11.22
ΣMUFA	22.55 ± 2.32	35.66 ± 5.55	46.63 ± 21.16	21.77 ± 6.21
ΣPUFA	32.75 ± 6.89	43.46 ± 5.64	40.45 ± 12.61	28.87 ± 4.43
Σn-3	24.72 ± 4.99	32.08 ± 4.33	30.72 ± 9.87	20.93 ± 3.33
Σn-6	1.99 ± 0.32	4.16 ± 0.59	4.34 ± 1.48	2.82 ± 0.59
(n-3)/(n-6)	12.29 ± 0.58	7.72 ± 0.11	7.18 ± 0.27	7.60 ± 0.78
ARA/DHA	0.08 ± 0.03 b	0.38 ± 0.01 a	0.39 ± 0.03 a	0.40 ± 0.07 a[*]
DHA/EPA	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00
% SFA	43.30 ± 3.53	46.48 ± 3.76	48.93 ± 4.04	43.67 ± 3.53
% MUFA	24.62 ± 2.42	25.31 ±2.53	26.56 ± 2.68	24.60 ± 2.44
% PUFA	32.07 ± 0.99	28.21 ± 0.86	24.50 ± 0.74	31.73 ± 0.95

1Moderate agitation; 2without agitation; 3vigorous agitation.
[*]Different letters across a row indicate significant difference ($n = 3$, $p < 0.05$, $b < a$)

4. Discussion

The strain of *M. moniliformis* was isolated, identified and evaluated in its potential of growth, the production of pigments and lipid and fatty acid, under controlled laboratory conditions. The strain was using different agitation levels, at 21 and 26 °C, and the growth and growth rates did not show significant differences. The results did not show difference between the agitation ways, indicating that the strain can be cultivated with or without agitation; this is also corresponding with the high tolerance of the strain that lives in agitated or quiet waters. Similar growth rates have been observed for other diatoms, such as *Cyclotella cryptica*, *Ditylum brightwelii, Skeletonema costatum, Chaetoceros simplex* (Montagnes and Franklin, 2001) and *Odontella aurita* (Pasquet *et al.*, 2014), suggesting high growth potential of *M. moniliformis* that in this case grew without a specific CO_2 supply. Additionally, Lorenz *et al.* (2005) mentioned that agitation is beneficial for some diatoms to get high densities; while others do not tolerate vigorous agitation, reducing its cell density and causing cell damage.

This diatom has widespread in marine and brackish water, is one of the species of the communities considered as the major food source for marine organisms (MacIntyre and Cullen, 1996; Woelfel *et al.*, 2014) that live in agitated water, sediments or surface waters, greatly contributing to primary productivity.

By size *M. moniliformis* is classified as microplankton, and as many other diatoms, pennate and centric, it is able of polysaccharide secretion that is a crucial part of their biological success (Underwood *et al.*, 2004). The polysaccharide production is possibly correlated to low nutrient content what is more probably when growth reaches stationary stage (Leandro *et al.*, 2003). For *M. moniliformis*, the polysaccharide secretion is probably what favors the formation of chains and cell aggregation, this secretion allows greater stability to the particles against hydrodynamic damage (Liu *et al.*, 2011). When cells aggregate it is also probable they produce self-shading, and so diminish light capture. This can in part explain the high content of pigments, especially chlorophyll *a*.

The harvesting of many nanoplankton strains require energy expenditures of 20–30% of total production costs (Chen *et al.*, 2010; Selesu *et al.*, 2016; Dickinson *et al.*, 2017).

In the case of *M. moniliformis*, the size and the production of bounding cells could represent an advantage for the harvest with the consequent saving in total culture costs.

Temperature is a critical variable that influence on metabolic process, in the case of many diatoms these coincides with the results obtained by Woelfel *et al.* (2014) with a strain of *M. moniliformis* isolated from the brackish southern Baltic Sea, where typical seasonal changes in water temperature range from –1°C to +28°C, and salinity from 8 to 24, that are different from those of Laguna de La Paz, B.C.S. with a warm dry desert climate, water temperature range of 17–33 °C, and salinity from 32–40 (Camacho-Mondragón *et al.*, 2012; Chávez-Sánchez *et al.*, 2017). Detained growth of *M. moniliformis* at temperatures ranging from 7–27 °C, salinity of 1–50, and irradiances of 10–600 μmol photon m^{-2} s^{-1}, this indicate the high adaptability of this diatom similar to others classified as eurythermic and euryhaline.

The content of chlorophyll *a* is a good indicator of the physiological status and microalgal growth, since it reflects the

state of the photosynthetic apparatus (Jiang *et al.*, 2011); a significant difference between treatments was not observed, indicating that all the conditions employed for cultivation were adequate for *M. moniliformis*.

In diatoms, chlorophyll *a*, *c*, and fucoxanthin form part of the fucoxanthin-chlorophyll-protein complex that replaces the harvesting complexes in plants or green algae (Gelzinis *et al.*, 2015) and function as accessory pigments in antenna complexes for harvesting light (Kuczynska *et al.*, 2015). Other carotenoids, such as *β*-carotene and xantophylls, diatoxanthin, diadinoxanthin, violaxanthin, anteraxanthin, and zeaxanthin function as protective pigments and participate in the xanthophyll cycle (Kuczynska *et al.*, 2015). Cultivation at 26 °C without agitation had less chlorophyll *c*, which may be related to a compensatory response to changes in lighting, since there was no agitation in this case. The lower total carotenoids productivity at 21 °C may be related to temperature. In both cases, further experimentation is necessary. Higher production of lipids at 26 °C without agitation may indicate some kind of stress, since the effect of nutrient deficiency, especially nitrates and silicates, is known; although this was not reflected in the total biomass production, possibly because the analysis was done at the end of the experiment. Again, this will require further experimentation.

Fatty acid composition of *M. moniliformis* is similar to other diatoms (Dunstan *et al.*, 1994; Falk-Petersen *et al.*, 1998; Courtois de Viçose *et al.*, 2012; Scholz and Liebezeit, 2013). There was high content of C16:0 and C16:1n7, and a variable but generally high content of C14:0 and C20:5n3, with relative deficiency of PUFA C18 and DHA C22:6n3, which is characteristic of diatoms. Some species could have high content of PUFA C16 and C18 (Scholz and Liebezeit, 2013). The highest content of fatty acids was of C16:2n3, C16:3n4, and C18:4n3, reaching mean values of 8.8%, 16.3%, and 6.3%, respectively, of the total. The fatty acid C16:4n3 was not detected, it is considered typical of lipids of some diatoms, probably because we cultivated at higher temperatures. This acid is responsible for increased membrane fluidity in cold-acclimatized cells (Jüttner, 2001).

5. Conclusions

M. moniliformis grew well under tested conditions, with no significant difference between conditions, and yielded high production of lipids and fatty acids. These results, together with the morphometric characteristics and their tendency to form chains, indicate that this species has high potential for lipid and fatty acid production in biotechnology.

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