Screening microalgae strains for their productivity in methane following anaerobic digestion

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Abstract: Interest in the use of microalgae for the production of biofuels has grown in recent years. Biomethane is a biofuel that can be obtained with high efficiency from anaerobic digestion of various organic feedstocks. In this study, a selection of freshwater (n=15) and marine (n=5) microalgae were tested in order to identify a microalgal strain that could be used as a model for large scale production of methane. Analysis of pH, volatile suspended solids and ammonium at the end of the assay ranged between 6.98- 7.66, 16.0- 25.9 g/L and 495- 1622 mg/L respectively. No significant differences in these values were detected between freshwater and marine strains. There was no significant difference in the methane yield from freshwater microalgae (329 ± 43 mLCH4/gTVS) and marine microalgae (298 ± 83 mLCH4/gTVS) although it varied greatly within the tested strains. A statistical analysis of the microalgae grown under two different culture media showed that the type of medium was more determinant than the type of microalgae (freshwater or marine) for the methane yield, with 310 ± 35, 365 ± 25 and 303 ± 77 mLCH4/gTVS for the freshwater microalgae grown in Bold's-3NV, f/2 and marine microalgae grown in f/2 media, respectively. The strains Scenedesmus sp.-AMDD, Isochrysis sp. and Scenedesmus dimorphus displayed the best methane yield with 410 ± 6, 408 ± 4 and 397 ± 10 mLCH4/gVS, respectively. The strain Scenedesmus sp.-AMDD was chosen as a model strain for future work development with continuously fed digesters.

February 13th, 2013

Dear Editor,

Please find enclosed a revised version #2 of the manuscript entitled "Screening microalgae strains for their productivity in methane following anaerobic digestion", that we wish to submit to Applied Energy. Each comment from the first revision was answered in a note written in blue, while modifications in the manuscript were written in red (manuscript marked). We also added a clear version of the revised manuscript. We like to thank again your reviewers for their efforts in such extensive and meticulous revision which certainly helped at improving the article. We hope that we were able to answer the reviewer concerns at his satisfaction.

Sincerely,

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_____

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Reviewers' comments:
There are still a few errors to be corrected before final acceptance.

The comments from the reviewer were all taken into considerations and modifications of the manuscript was made accordingly.

Reviewer #1:
1. Abstract line 29 please give range for quoted values of specific methane yield

The range was added in the abstract as requested.

2. Line 39 The statement that micro-algae offer higher areal biomass productivity is contentious as this has yet to be demonstrated on a year-round basis at large scale. It should be qualified in some way, e.g. by adding the word 'potentially' here or in the preceding line.

We agree with the reviewer that higher areal biomass productivity is still not fully demonstrated and we added "potential" before "advantages" as requested (line 35).

3. Lines 77-83 Phaeodactylum tricornutum was not fully degraded under the conditions applied, but this does not mean that the only possible approach is to look for a strain which degrades better. The points in this section are not well expressed.

The authors did not intend to assert that the only approach to partial degradation of an algal strain is to look for a strain that can be better hydrolyzed. However, we believe that looking for a strain that can be degraded and converted into methane at a higher rate is certainly a valid approach. The purpose behind finding a better strain was clarified in the text (optimize biofuel production per kg of initial biomass).

4. Lines 128-9 The samples were received and tested over a 2-year period. Was any positive control used in the BMP test procedure to ensure that the results are comparable? This may be specified in the test protocol, but should be stated here as the reference given is not readily available.

There is no positive control *per se* for a BMP test. The protocol specifies that an inoculum containing active anaerobic biomass should be used for the digestion of the tested substrate. In our case, granulated sludge was periodically collected at a full-scale anaerobic digester to start the different series of incubation. This digester is operating with great stability for the past 20 years on the same substrate. While it is not possible to know the exact composition of the inoculum (it is a consortium of several hundreds of bacterial species), this inoculum was also used in our laboratory for other purposes and its fermentative and methanogenic activities were measured and maintained over time. A line was added in the manuscript to add this information (lines 138-140).

5. Line 155 The method used for gas collection means that the headspace is pressurized and it is likely that some carbon dioxide remained dissolved in the digestate liquor. As the methane yield only is reported this should not greatly affect the results, but it should be noted that this can affect the conditions of the test itself.

Thank you for the comment. Indeed, there is an equilibrium between dissolved CO_2 and gaseous CO_2. Our modified BMP test is performed in bottles with large headspace (over 400 mL) and we collect the biogas production regularly to minimize the pressurization of the biogas in the headspace.

6. Lines 191 - 194 This covers the same points as lines 198-200 but is incorrect and looks as if it may have been left in from an earlier revision.

The reviewer is correct and the lines were removed from the manuscript.

7. Line 202 Results are reported in terms of VSS, but no method is given in the section on analytical methods. Is the use of VSS = volatile suspended solids correct, or should this be e.g. total volatile solids as in the methods section? VSS is also used in the introduction (line 73) before it has been defined.

The analysis performed at the end of the incubation for each test bottles included VSS (volatile suspended solids). The methodology number was added in section 2.3. Also, VSS was defined in the introduction.

8. Lines 216-229 The statement made in lines 221-224 cannot be supported by the experimental methodology used, for the reasons given by the authors themselves in lines 224-226. If the average concentration of ammonium at the end of the assay was 883 mg/l and the quantity of micro-algae added was 1 g VS in 100 ml, it would be just as reasonable to argue that the ammonium released into the medium represents 8.8% of the total algal VS. This in turn may represent quite a high degree of protein hydrolysis, based on typical literature values for the nitrogen content of algal biomass. Without knowing the nitrogen content of the samples tested it is difficult to say either way. The authors also do not tell us the ammonium content of the inoculum-only control sample at the end of the test, which would provide a useful comparison - although it could be argued that more ammonium might be released by the inoculum-only controls if unfed inoculum biomass has died and cell lysis has occurred. It is generally quite difficult to deduce anything from the nutrient concentration at the end of a batch test of this type especially where the amount of inoculum VS added is greater than that of the sample, as in the present case. The authors either need to make a much stronger quantitative case to support their statement, e.g. based on mass balance and comparison, or to delete the sentence in lines 221-224 and re-write the rest of the section.

The reviewer is correct about conflicting statements of lines 221-224 and 224-226. Most of the revised manuscript section was added during the first revision of the manuscript. The ammonium concentration were measured mostly to insure that no

inhibitive concentrations were found at the end of the assays and a discussion more focused to that point is provided in revision #2. The average concentration of ammonium measured at the end of the assays for the controls was added in the manuscript (lines 216-224).

9. To avoid confusion over units when considering the breakdown of organic nitrogen-containing materials, ammonium is often reported in terms of its nitrogen content. Can the authors clarify whether the ammonium concentrations quoted are in mg NH4/l or mg N/l? This applies throughout the paper including tables

The authors expressed their results in mg NH_4/L, and this was specified in the methodology section (lines 175-176).

10. Line 314 The methane production from three Chlorella strains ranged from 263 to 302 mL CH4/gVS. The highest methane production from Chlorella appears to be 361 mL CH4/gVS, with 309 and 331 CH4/gVS from Chlorella sp. -RB1a and Chlorella sorokiniana. If there is a reason for selecting the other values and omitting these, this should be made clear here.

The authors were simply suggesting an hypothesis for the low methane production values for some of the *Chlorella* strains tested. The sentence was modified in the manuscript to better reflect the author's intention (lines 307-310).

11. Line 342 - 348 In statistical terms, the comparison is not meaningful due to the low number of replicates: the tests used are unsuitable for triplicate results with such wide variability. Equally importantly, the difference between the mean values for the two samples is 28 ml CH4/gVS. This difference in methane yields is large enough in itself to be of commercial significance; the average values for three other species (Porphyridium aeruginosa, Micractinium and Chlorella vulgaris) lie between those for the two B. braunii samples. This is not a criticism of the results themselves: carrying out replicated comparative tests of this type is difficult and very demanding in terms both of materials and of equipment, and in general the agreement between triplicates is good - it is unfortunate that the first B. braunii sample has a slightly higher relative standard deviation than most of the others. But unless the authors are implying that the difference between 342 and 370 mL CH4/gVS is itself of no importance, the data here only confirm that yields may vary for a number of reasons. They cannot be used to support the argument that the methane yields are similar in similar conditions, and this section should therefore be modified or deleted.

The authors thank the reviewer for the comment and his acknowledgment of the work carried in this study and inherent variability of the test. As this study is a preliminary screening at a modest scale, the authors are not convinced that commercial argument can be made at this point. In the case of *B. braunii*, the 28 ml CH4/gTVS did not result in a statistical significant difference in the methane production between the 2 set of triplicates. At this point of the investigation, the authors do not think that too much

focus should be put on the difference between 342 and 370 mL CH4/gTVS, as the final objective is to identify the most promising algae strain and clearly *B. braunii* does not meet that objective, either at 342 or 370 mL CH4/gTVS, If the statistical analysis is discarded due to the low number of replicates, it is believed that the difference in methane production between the two sets of samples should not be considered significant either. The manuscript was modified to remove the statistical aspect of the discussion as requested by the reviewer (lines 335-340).

12. Line 367 - 371 This seems to conflict with the newly added lines 363-365 - has something been inadvertently left in the revised text?

The reviewer is correct and the lines were removed from the manuscript.

13. Lines 423 - 437 This section needs to be moved to the end of section 3 Results and discussion, as it is not a conclusion from anything that has been presented in the paper so far.

The reviewer is correct and the lines were moved to a new section 3.5 of the manuscript (lines 391-406).

14. Table 2 and 3 Names of microalgal species are inconsistent and in some cases misspelled e.g. Porphyridium aerugineum /Phorphyridium aeruginosa, Thalassiosira weisfloggi/ Thalassiosira weissflogi)

The authors thank the reviewer for spotting these accidental misprints. The names of the microalgal species were corrected in the Table.

15. The text is full of small grammatical errors and turns of phrase that could easily be corrected by a native English speaker.

The text was revised as requested and the authors hope that it is now in an acceptable form.

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Screening microalgae strains for their productivity in methane following anaerobic digestion

Highlights

- There were no significant differences in the methane potential from freshwater or marine microalgae.
- Freshwater microalgae showed higher methane production when cultured in f/2 medium compared to Bold's 3N.
- Methane production of up to 408 mL CH_4/gVS could be achieved when anaerobically digesting microalgae.
- The strain *Scenedesmus* sp. AMDD was chosen as a model strain for future work.

Screening microalgae strains for their productivity in methane following anaerobic digestion

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ABSTRACT

Interest in the use of microalgae for the production of biofuels has grown in recent years. Biomethane is a biofuel that can be obtained with high efficiency from anaerobic digestion of various organic feedstocks. In this study, a selection of freshwater (n=15) and marine (n=5) microalgae were tested in order to identify a microalgal strain that could be used as a model for large scale production of methane. Analysis of pH, volatile suspended solids and ammonium at the end of the assay ranged between 6.98- 7.66, 16.0- 25.9 g/L and 495- 1622 mg/L respectively. No significant differences in these values were detected between freshwater and marine strains. There was no significant difference in the methane yield from freshwater microalgae (329 ± 43 $mLCH_4$/gTVS) and marine microalgae (298 ± 83 $mLCH_4$/gTVS) although it varied greatly within the tested strains. A statistical analysis of the microalgae grown under two different culture media showed that the type of medium was more determinant than the type of microalgae (freshwater or marine) for the methane yield, with 310 ± 35, 365 ± 25 and 303 ± 77 $mLCH_4$/gTVS for the freshwater microalgae grown in Bold's-3NV, f/2 and marine microalgae grown in f/2 media, respectively. The strains *Scenedesmus sp.*-AMDD, *Isochrysis* sp. and *Scenedesmus dimorphus* displayed the best methane yield with 410 ± 6, 408 ± 4 and 397 ± 10 $mLCH_4$/gVS, respectively. The strain *Scenedesmus* sp.-AMDD was chosen as a model strain for future work development with continuously fed digesters.

KEYWORDS

anaerobic digestion; methane; microalgae; biofuel; bioenergy; Scenedesmus

1. INTRODUCTION

There is a growing interest in the use of microalgae for the production of biofuels in recent years [1], as algal biomass offers several potential advantages compared with other feedstocks, including higher areal biomass productivity, high lipid content and higher value products [2]. Although past efforts were mainly engaged in the development and processing of microalgae strains for the production of biodiesel [3, 4], conversion of algal biomass into biomethane is drawing increasing attention [5, 6]. The use of the whole microalgae for methane production as a biofuel has been suggested and verified in a life cycle analysis (LCA) [7], which showed that methane compares favourably with other biofuel production scenario. Although it is not yet clear what the most effective process for biofuel production from microalgae is, anaerobic digestion and methane production is certainly the least complex one [5]. Some authors are more assertive, and suggest that the production of methane via anaerobic digestion (AD) is the most feasible and cost-effective route to an energy product [8]. This is supported by Harun et al [9] who demonstrated that more energy could be generated from the production of methane from microalgae (14.04 MJ/kg), rather than biodiesel (6.6 MJ/kg) or ethanol (1.79 MJ/kg) where their unit "kg" is assumed to be "kg of dry weight algae". Furthermore, up to 65% of the chemical energy stored in the algal biomass can be potentially recovered through AD to methane [10].

Anaerobic digestion is already successfully applied to the conversion of a wide variety of organic substrates to methane, such as the organic fraction of municipal solid wastes [11], waste activated sludge [12], and energy crops [13]. Recent studies are increasing our knowledge about anaerobic digestion of microalgae. Theoretical calculations [14] as well as bottle and digester experiments [15] have shown the great potential of anaerobically digesting microalgae for methane production which can be further converted into a clean and renewable biofuel.

Microalgae macromolecular distribution and cell walls renders anaerobic digestion efficiency strain –specific [5]. This was emphasized by Mussgnug et al. [15] who suggested testing strains individually since their methane potential could not be inferred

from their phylogenetic classification. Biomethane potential assays were performed on microalgal biomass, and showed a wide spectrum of methane yields. For example, Zamalloa et al. [16] showed 0.36 and 0.24 L CH_4 g^{-1} volatile solids (VS) for *Phaeodactylum tricornutum* and *Scenedesmus obliquus,* respectively. A conversion efficiency of 51% was obtained from *P. tricornutum* in a continuous digestion in a hybrid flow-through reactor. A similar performance was observed during the digestion of *Chlorella vulgaris* in a 1L digester at 24d HRT, where 51% inlet COD degradation for 240 mL CH_4/g volatile suspended solid (VSS) added was obtained [17]. Fed-batch assays confirmed that the limiting step for algae digestion was the hydrolysis. Recent studies performed in an anaerobic membrane bioreactor with *P. tricornutum* have confirmed that around 50% of the tested microalgal biomass was not degraded into methane [18], thus emphasizing the interest in identifying a potential strain more easily hydrolyzed thus yielding more methane per kg VS, i.e. a higher biofuel production per kg of initial substrate. Alternatively, high lipid content would theoretically improve the methane potential of whole microalgae. However, the cultivation parameters involved (high light intensity, nutrient starvation for example) which would increase the accumulation of lipid in the cells, would come at the expense of microalgae biomass productivity. It is not clear if this particular cultivation mode would result in a higher methane yield and an optimal scenario for microalgae biomass and lipid productivity has still to be determined.

Fermentation of marine microalgae could be inhibited because of high levels of sodium [14]. However, it seems that marine algae are more prone to disintegration when mixed with anaerobic fermenter sludge [15] resulting in the release of more intracellular material which could theoretically enhance methane production. It is not clear which species of freshwater or marine microalgae would be best suited for optimal methane production.

Although there have been recent developments in the field of biomethane production from microalgae, there is still a need to screen multiple strains to identify one that could combine as many of the desired traits as possible: ease of cultivation, high biomass

yields, high protein and/or lipid content and ease of anaerobic biodegradation. The purpose of this study was thus to evaluate the methane potential from a selection of freshwater and marine microalgae grown on two culture media. The final objective was to identify a microalgal strain that could be used as a model for future work and upscaled experiments for biomethane production.

2. MATERIALS AND METHODS

2.1 Growth and culture conditions

The freshwater strains *Neochloris oleoabundans*, *Chlorella vulgaris*, *Scenedesmus dimorphus*, *Porphyridium aerugineum* and *Botrycoccus braunii* were obtained from the University of Texas Culture Collection (strain ids 1185, 265, 1237, 2618 and 572, respectively). The other freshwater strains used in this study but not obtained from the UTEX collection were isolated from the Canadian province of Saskatchewan as described in Park et al. [8]. Some of these strains including *Scenedesmus sp*.-AMDD, *Scenedesmus sp*.-PN2, *Chlamydomonas debaryana*-AMB1, *Chlamydomonas sp*.-AMLS1b, *Chlorella sorokiniana*, *Chlorella sp*. Island-R, *Chlorella vulgaris* and *Micractinium sp*.-RB1b were isolated from soil samples. All of these isolates were photoautotrophically cultivated in Bold's-3NV (B3NV) medium as shown in Table 1 [8]. The marine strains *Phaeodactylum tircornutum*, *Nannochloropsis gaditana*, *Thalassiosira weisflogii*, *Glossomastix chrysoplasta* and *Isochyrsis* spp. (strain ids 1327, 525, 1336, 1537, and 462, respectively) were obtained from the National Centre for Marine Algae and Microbiota (formerly the Provasoli-Guillard Culture Collection of Marine Protozoa), East Boothby, Maine. All marine strains were cultivated in Pasteurized seawater in f/2 media [19] as detailed in Table 1. Table 2 shows the different strains tested for their methane potential along with the specific medium in which they were cultivated and their total solids (TS) content after harvesting by centrifugation and total volatile solids (VS) after combustion. The microalgal biomass was collected by centrifugation (CEPA Z101 process centrifuge; 15,000 x g) at a processing rate of 20 L/min for a typical duration of 30 minutes. Table 2 also lists the strains as either freshwater or marine.

2.2 Preparation of the methane potential assays

Biomass samples of the microalgae strains listed in Table 2 were received and tested between September 2009 and November 2011. The methane potential assays were prepared based on the Biochemical Methane Potential (BMP) assay for wastewater [20]. A few modifications were made to adapt the test to high solid samples [21]. The assays were performed using an inoculum to microalgae ratio of 2:1, based on the VS concentration, to ensure better kinetic constants [22]. The inoculum consisted of 20 g of granular biomass (wet weight) collected from a full scale upflow anaerobic sludge blanket (UASB) digester treating apple processing wastewater (Lassonde Inc., Rougemont, QC, Canada; 45°25'52.71" N, 73°03'12.15" W), with a moisture content of 90%. The inoculum was starved for 48 hours prior to the start-up of the assays, by incubation at 35°C and at agitation at 150 rpm with no substrate. Altough the assays were performed at different times over the two year period of this study, the methanogenic activity of the inoculum was maintained over time.

Triplicate bottles (500 mL) were prepared anaerobically under a constant flow of a gas mix (80% N_2, 20% CO_2) for each experimental digestion. Before sealing, the pH was adjusted to 7.0, if necessary, when the bottles were ready. A typical bottle contained one gVS of the tested microalgae, 2 gVS of inoculum, two mL of defined media, two ml of bicarbonate buffer and 0.5 ml of 1.25% Na_2S-cysteine solution. The recipes for the different solutions and the procedure for their preparation are detailed elsewhere [21]. The final volume was adjusted to 100 mL for all bottles using boiled demineralized water. The bottles were incubated at 35°C with an agitation of 150 rpm. Control bottles were prepared to correct for endogenous methane production from the assays. The control bottles were identical to the test bottles, excepted that the microalgal suspension was replaced with the same volume of deoxygenated water. The assays were conducted until the methane production became negligible (< 3 ml d^{-1}) which typically occurred between 34 and 50 days of incubation.

2.3 Analytical methods

The biogas production was released from the bottles at regular interval, generally four times in the first week of incubation, and twice weekly afterward, using a water-displacement system built from a volumetric glass burette, graduated every 0.2 ml. The bottles were allowed to equilibrate by displacing water from the burette to a connected Erlenmeyer flask, which required around 20 seconds to perform. A gas sample (0.3 ml) was then taken from the headspace of the bottles using a model 1750 gas-tight syringe (Hamilton, Reno, USA) and analyzed for H_2, N_2, CH_4 and CO_2 by gas chromatography (GC) as described in Frigon et al [21]. All gas or methane volumes presented in this study are described at standard temperature and pressure, of 273.15 K and 100 kPa pressure.

A few parameters were monitored on the algae paste and at the end of the incubation for each set of assays, including total solids (TS), total volatile solids (VS), volatile suspended solids (VSS), pH, soluble chemical oxygen demand (sCOD), ammonium (NH_4) and volatile fatty acids (VFA), namely acetate, propionate and butyrate. The pH was measured on an Accumet AP61 portable pH meter equipped with a micro probe (Fisher, Fairlawn, USA) directly on the recovered sample, within one minute of sampling. The TS, VS, VSS and sCOD concentration were determined according to Standard Methods [23] using methods 2540B, 2540D, 2540E and 5220D, respectively. The ammonium and VFA were analyzed by GC [21]. The ammonium concentration was expressed as mg NH_4/L throughout the manuscript.

2.4 Statistical analysis

As a first step, the homogeneity of variance was tested using Levene's F-test [24]. This test provides a significance value (P-value). If P is greater than the significance level of 0.05 (alpha), the group variances can be treated as equal. Otherwise ($P < 0.05$), we have unequal variances. Then a Student's t-test was performed to determine whether there was a statistically significant difference between the means in the two groups when variances were equal. Otherwise, a Welch's t-test was used [25]. In both t-tests, the means from the two unrelated groups were considered as not significantly different (null hypothesis) when the P-value was greater than the significance level of 0.05 (alpha). All statistical

tests were performed using Microsoft Excel (Microsoft Corporation, Redmond, Washington).

3. RESULTS AND DISCUSSION

3.1 Results for the physico-chemical parameters for all methane potential assays

Methane potential assays were performed in triplicate for 15 freshwater and 5 marine microalgae strains. Table 3 presents the results obtained at the end of the incubation period for all tested strains. The pH was measured at the end of each assay and varied between 6.98 ± 0.03 and 7.66 ± 0.05. In parallel to a neutral pH, there was no VFA accumulation at the end of the incubation period for almost all of the assays reported. The VFA concentration was low for the two strains with reported VFA, *B. braunii* Mar-2010 and *G. chrysoplasta*. The neutral pH and the absence of VFA accumulation are thus indications that no irreversible inhibition occurred and conditions were satisfactory by the end of the test.

Table 3 presents results for the volatile suspended solids (VSS), sCOD and VFA concentration obtained at the end of the methane potential assays. Although it can be presumed that a high level of microalgae degradation in the assays would result in lower VSS concentration at the end of the incubation, there were no strong correlation between the final VSS concentrations in the assays and the methane production for either the freshwater (coefficient of correlation (R = 0.322) and marine (R = 0.535) microalgae. This could be related to the high amount of inoculum which contributed to 2/3 of the initial VSS content in the bottles. The final soluble COD concentration can give an indication of the amount of substrate hydrolyzed but recalcitrant to further mineralization toVFA and then methane and CO_2. The sCOD concentration were low in general for the freshwater strains but rather high for the marine strains. It can be presumed that the high sCOD concentration represented recalcitrant or non biodegradable material.

The average final ammonium (NH_4) concentration was 351 ± 16 mg/L in the control bottles containing only inoculum. Not including *Isochrysis sp.*, an average final

ammonium concentrations of 883 ± 140 mg/L was observed for all tested strains. The final ammonium concentration in digestions containing *Isochyrsis sp.* was 1622 ± 105 mg/L, which was considered an outlier. These concentrations were well below those considered inhibitory [26]. These values are further indications of the proper conditions in the assays, and shows that the digestion of the microalgae at low initial VSS concentration would greatly reduces the potential of ammonia for feedback inhibition on methanogenesis as reported by Heaven et al. [27].

The variances of the average pH, VSS, sCOD and ammonium concentrations for the freshwater and marine microalgae were compared, followed with a t-test, in order to determine whether the means of the physico-chemical parameters were significantly different for the two groups of microalgae (Table 4). The resulting P values were 0.270, 0.151, 0.035 and 0.381 for pH, VSS, sCOD and ammonium, respectively. Therefore, there were no significant differences between the final pH, VSS and ammonium concentration between the freshwater and marine microalgae at the end of the digestion assays. However, the sCOD concentration were significantly higher in the case of the marine microalgae, except for *N. gaditana*.

3.2 Overview of the methane production for all assays

The methane production for all tested strainsvaried between 227 -410 mL CH_4/gTVS. Representative time-courses showing the kinetics of methane accumulation from digestion of five microalgae strains over time are shown in Figure 1. The onset of methane production appeared to take place without delay in the assays, probably due to the fast initial transformation of soluble biodegradable matter. Initial methane production (day 0 – 3) was significantly different for the tested strains and reached 19, 23.5, 23.3, 23.8 and 37.5 mL $CH_4/gVSS_{inoculum}$.d for *C. sorokiniana*, *Chlorella sp. Island-R*, *C. debaryana-AMB1*, *C. sp.-AMLS1b* and *Micractinium sp.*, respectively. While *Micractinium sp.* displayed the highest initial methane production for all tested strains, *Thalassiosira weisflogii* showed the lowest methane production at 8.3 mL $CH_4/gVSS_{inoculum}$.d. This could be due to the presence of the silica frustules which might

have impeded digestibility. *Micractinium sp.*, which showed the highest initial methane production, also yielded more methane than the other strains from Figure 1.

A decrease in the methane production kinetic was observed after the first four days in almost all of the anaerobic digestion assays performed in this study, and persisted until days 14 to 17 of incubation. This latency could be related to high lipid content and partial inhibition from long chain fatty acids (LCFA) [28]. However, the inflection in the methane production kinetic could be caused more simply by a physical barrier such as potentially recalcitrant algal cells impeding hydrolysis and preventing the release of soluble biodegradable compounds. There was no such inflection in the methane production for *Micractinium sp.* (Figure 1), as well as in *Scenedesmus dimorphus*, *Isochrysis sp.*, *Glossomastix chrysoplasta* and SK-RB1a (data not shown). *Isochrysis sp and Scenedesmus dimorphus* had the 2nd and 3rd highest final methane yields out of 20 strains tested.

It is possible to estimate a theoretical methane yield from microalgae biomass based on an average elemental formula of $C_{2.11}H_{3.93}ON_{0.26}$ [27]. The maximal CH_4 yield would then be 0.55 Nm^3 CH_4/kgTVS, although this can probably not be achieved in practice due to recalcitrant material that is always present in any organic matter. This generic stochiometric value could also underestimate the methane yield achievable from lipid-rich microalgae. For instance, microalgae containing 40% lipids, 20% carbohydrates and 28% proteins would have a theoretical yield as high as 0.68 Nm^3 CH_4/kgTVS. This highlights the importance of using actual assayed values of methane production from algal biomass rather than theoretical estimates. The final methane production from *Scenedesmus* sp.AMDD-Jul 2011 and *Isochrysis spp.* represented between 60 and 75 % of the theoretical methane yields predicted from the high-lipid and average elemental stochiometries mentioned above, respectively.. The relatively high methane yields obtained from *Scenedesmus sp.*-AMDD and *Isochrysis sp.* indicates they may be good candidates for large-scale production.

Previous studies have discussed the similarities and differences of microalgal biomass and waste activated sludge (WAS) regarding their composition and anaerobic

degradation potential [17, 29]. In this study, it was shown that methane production from microalgae was a relatively fast process, with digestion times that were comparable to what is required for municipal sludge (20-40 days) [30]. The methane yield was over 330 Nm^3 CH_4/kgTVS for 50% of the microalgae strains tested (Table 1), representing a conversion efficicency of 60% using the stochiometric formula detailed above, and this would suggest better amenability to biodegradation than WAS [12].

One factor that could have contributed to the high methane yields obtained in this study could be the freezing of the microalgae paste for storage prior to shipment between collaborating laboratories. This can be considered a form of pretreatment that may to some extent disintegrate the microalgae prior to digestion. Freeze thaw cycling is known to cause a decrease in the volatile solids (TVS) of mixed sewage sludge simultaneously with an increase of the soluble COD and VFAs, thereby improving biogas yield [31]. This is consistent with the results of Harith et al [32], who showed that freezing the marine diatom *Chaetoceros calcitrans* at -20°C for 2 weeks decreased its viability upon thawing. Another positive aspect of the present study is our use of wet algal biomass. The use of dried algae biomass has been shown to reduceits digestibility compared to wet material [33].

Some of the microalgal strains tested in this study have been reported to contain high oil content (% dry wt): *Botryococcus braunii* (25-75), *Chlorella* (28-32), *Isochrysis sp.* (25-33), *Nannochloropsis sp.* (31-68), *Neochloris oleoabundans* (35-54), *Phaeodactylum tricornutum* (20-30) (extracted from Table 2 in [4]). Their methane production ranged from very low (228 mL CH_4/gTVS for *Nannochloropsis gaditana*) to high (408 mL CH_4/gTVS for *Isochrysis sp.*, Table 3). The low yield obtained for *Nannochloropsis* could be related to its tough cell wall, caused by the presence of sporopollenin polymers [34]. The high methane production from the digestion of *B. braunii* (343-370 mL CH_4/gTVS) could be due to the presence of an external lipid biofilm matrix thatholds the fan-shaped colonies of *B. braunii* together [35]. Six different strains of *Chlorella* were tested in this screening study and their methane yields were lower than in previous reports, except for *C. vulgaris* at 361 ± 11 mL CH_4/gTVS, possibly due to their

311 recalcitrant cellulosic cell walls [36]. Among the strains listed above, *Isochrysis sp*.
312 showed the highest methane production (408 mL CH_4/gTVS). *Isochrysis sp*. is known to
313 synthesize high amounts of lipids, mainly polyunsaturated fatty acids (PUFAs) [37].
314 Furthermore, the absence of a tough cell wall makes this strain an interesting prospect for
315 biofuel production.
316
317 The highest methane yield (410 ± 6 mL/gTVS_{in}) was obtained with wastewater-grown
318 *Scenedesmus* sp.-AMDD, despite previous reports that *Scenedesmus* are supposed to be
319 highly recalcitrant to digestion due to a tough polysaccharide-based cell wall [15, 38].
320 This is in contrast to the findings of Mussgnug *et al* [15] where a relatively low methane
321 yield of 287 mL/gVS was reported from *Scenedesmus obliquus*. Light microscopy photos
322 even showed intact cells after prolonged anaerobic incubation, and their hypothesis for
323 methane production within the digester included methane from debris transferred with the
324 culture or biodegradable metabolites provided by the activity of *Scenedesmus* within the
325 digester. Presumably, the specific inoculum used in our BMP assays had a stronger
326 cytolytic activity than inocula from other studies. A higher cellulase activity in the assay
327 would favor the disruption of the cell wall and membrane of the microalgae [39], thus
328 allowing a higher methane production.
329
330 However, the *Scenedesmus sp.*-AMDD strain yielded significantly less methane (306 ±
331 14 mL CH_4/gTVS) when growing in the Bold's 3N medium as compared with
332 wastewater. In a related study, an average methane production yield of 340 mL/gTVS
333 (for a 56% conversion efficiency) was obtained with *Scenedesmus sp.*-AMDD grown on
334 a different municipal wastewater [10]. The difference observed for the three experiments
335 with *S. sp*-AMDD supports the view that factors such as the culture medium and growth
336 conditions could have a significant impact on the specific methane yield. Methane yields
337 from digestions of specific algae strains grown in the same medium are generally less
338 variable than when grown in different media. For instance, the methane production from
339 two *Botryococcus braunii* assays grown in f/2 medium fifteen months apart reached 342
340 ± 23 and 370 ± 10 mL CH_4/gTVS, respectively.
341

342 3.3. Comparison of the methane production results from the freshwater and marine strains
343 One of the objectives of this study was to compare the methane production potentials
344 obtained from freshwater versus marine microalgae. It is interesting to note that both
345 freshwater (*Scenedesmus sp.*-AMDD, 410 mL CH_4/gTVS) and marine (*Isochrysis sp.*,
346 408 mL CH_4/gTVS) microalgae have the potential to generate high yields of methane
347 after anaerobic digestion. Figure 2 presents the methane produced for all screened strains,
348 grouped between freshwater and marine microalgae. The average methane production
349 from the freshwater microalgae was 329 ± 43 mL CH_4/gTVS, compared with 298 ± 83
350 mL CH_4/gTVS for the marine strains. It can be clearly seen from the size of the boxes
351 and the standard deviations, that the methane production varied greatly, in particular for
352 the marine strains. The data from both groups were processed through an F-test resulting
353 in unequal variances (P = 0.027), followed by a t-test showing no significant difference
354 (P = 0.229) between the methane yields obtained from freshwater or marine microalgae.
355 The choice of a microalgal strain for methane production will therefore have to be made
356 considering the different aspects of the culture of the model strain (productivity, use of
357 land, harvesting).
358
359 3.4. Comparison of the methane production results as a function of the cultivation
360 medium
361 All the marine strains tested in this study, along with four freshwater strains, were grown
362 on f/2 medium. Figure 3 shows the methane production results, grouped with respect to
363 the growth medium, and with a further separation between B3NV and f/2 media for the
364 freshwater strains. The average methane production from the freshwater microalgae was
365 310 ± 35 and 365 ± 25 mL CH_4/gTVS with B3NV and f/2 media, respectively. The
366 average methane production for the marine microalgae grown on f/2 medium reached 298
367 ± 83 mL CH_4/gTVS. As mentioned in Section 3.3, the methane production seemed to
368 vary more for the marine strains.
369
370 The three groups of data were processed through an F-test for variance, followed by a t-
371 test assuming equal / unequal variances to evaluate if their means were equal or
372 statistically different, as reported in Table 5. The statistical analysis was performed using

the average values from the triplicates, i.e. performed on 12, 4 and 5 values for the B3NV and f/2 media for freshwater microalgae and f/2 medium for marine microalgae, respectively. There was a significant difference (P = 0.004) in the methane production results for the freshwater strains between B3NV and f/2 media. A comparison between B3NV and f/2 medium revealed that the B3NV medium is significantly richer in nutrients with 10 times more nitrates and 47 times more phosphates (Table 1). The f/2 medium could have promoted the accumulation of lipids in the algae strains which would have resulted in higher methane production after anaerobic digestion (Figure 3). B3NV medium also contained much more cobalamin (vitamin B12). However, the exact role of cobalamin in the microalgae metabolism is still unknown and around half of the microalgae species can synthetize their own cobalamin [40]. Therefore the potential benefits of a higher cobalamin dose could not be confirmed as the capacity of each of the tested micro-algae for B_{12} synthesis is unknown.

There was also a significant difference (P = 0.036) in the methane production results for the freshwater and marine strains grown on f/2 media, although the low number of samples from which the means were obtained could limit the statistical significance of the test.

3.5. Cost aspects of producing methane from microalgal biomass

A recent cost analysis [41] concluded that methane production and cogeneration from microalgal biomass would become profitable from a feed-in tariff (FIT) of €0.133/kWh for both heat and electricity on an equal basis and a carbon credit of €30/t eCO_2, although the latter would only represent 4% of the revenue. The analysis assumed that the algal culture in raceway ponds can have a minimal productivity of 90 dry t/ha.yr, be concentrated up to 20–60 dry kg/m^3 at the harvest, which is estimated to represent a feedstock cost of €86–€124/dry t, and that the algal concentrate can be processed in an anaerobic reactor at a loading rate of 20 kg VS/m^3·d with a conversion efficiency of 75%. Our results show that a number of microalgal strains have a methane potential near or above 0.4 Nm^3/kg VS (i.e. corresponding to a conversion efficiency of ca. 75%), which would match or even lower the minimum FIT for profitability in the above case study. A

variety of pre-treatment techniques could certainly improve the methane production from microalgal biomass, and accordingly increase the revenue [6]. But the addition of a pre-treatment stage would also increase the capital and operation costs, which may not be offset by the gain in methane.

4. CONCLUSIONS

The identification of a particular microalgae strain as a model for biofuel production represents a challenge considering that many parameters such as high biomass and lipid yields, which are often mutually exclusive, have to be taken into account. The approach that was favored in this study was to target strains with a high dry weight to culture volume ratio.

In this study, a screening of the methane production potential of freshwater and marine microalgae was performed in order to identify the most promising strain for further work development. Specifically, the highest methane production was obtained from *Scenedesmus dimorphus*, *Scenedesmus sp.* AMDD and *Isochrysis sp*., among the 20 tested strains. Some interesting outcomes were derived from these assays, such as the demonstration that high methane production can be obtained from previously reported hard to digest microalgae strains, without any preliminary pretreatment aside from the potential impact of freezing / thawing, with unadapted anaerobic inoculum. Also, the impact of the growth medium on the resulting methane production from the microalgae was shown to be significant, independant of the type of water in which the microalgae are grown.

Among the three highest methane yielding strains, *Scenedesmus sp.* AMDD was chosen for further study, for practical reasons, as it is robust, easy to cultivate and generates high biomass yields on municipal wastewater. Future work will include continuous digestion of microalgal biomass in lab-scale digesters, and the use of thermal and chemical pretreatments in order to increase the methane production.

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554
555

Figure captions

Figure 1. Typical time courses of methane production from anaerobic digestion of five microalgae strains. The cumulative methane production for each of the strain is expressed in mL of methane produced per gram of total volatile solids of microalgae added in the test bottles. The methane production shown is a net production, e.g. with endogenous control removed.

Figure 2. Comparison of the amount methane produced from freshwater versus marine microalgae strains. The methane production for each category of microalgae is expressed in mL of methane produced per gram of total volatile solids of microalgae added in the test bottles. The box plot can be described as follow: the lower and upper limit of the box represents the lower (25%) and upper quartile (75%) for the data distribution. In other words, 50% of the methane production values are comprised within the box. The line inside the box represents the median value (50%). The whiskers represent the minimum and the maximum values for each category of microalgae.

Figure 3. Comparison of the amount methane produced from the microalgae strains as a function of the culture growth medium. The methane production for each category of microalgae is expressed in mL of methane produced per gram of total volatile solids of microalgae added in the test bottles. The box plot can be described as follow: the lower and upper limit of the box represents the lower (25%) and upper quartile (75%) for the data distribution. In other words, 50% of the methane production values are comprised within the box. The line inside the box represents the median value (50%). The whiskers represent the minimum and the maximum values for each category of microalgae.

583 Table 1. Comparison between the composition of the Bold's 3N and f/2 media
584

Compound	Bold's 3N (mM)	f/2 (mM)	Ratio Bold/f2
NaNO3	8.82	0.882	10
$FeCl_3 \cdot 6H_2O$	$2.16\ 10^{-3}$	$1.202\ 10^{-2}$	0.2
$MnCl_2 \cdot 4H_2O$	$1.26\ 10^{-3}$	$8.843\ 10^{-4}$	0.1
Zinc chloride / sulfate 84%	$2.22\ 10^{-4}$	$7.826\ 10^{-5}$	2.8
$CoCl_2 \cdot 6H_2O$	$5.04\ 10^{-5}$	$4.203\ 10^{-5}$	1.2
$Na_2MoO_4 \cdot 2H_2O$	$1.02\ 10^{-4}$	$3.640\ 10^{-5}$	2.8
$Na_2EDTA \cdot 2H_2O$	$1.02\ 10^{-2}$	$1.142\ 10^{-2}$	1.1
Sodium phosphate	1.72	$3.623\ 10^{-2}$	47
Vitamin B12	$1.0\ 10^{-4}$	$3.687\ 10^{-7}$	271
CaCL2.2H2O	0.17	N/A	N/A
MgSO4.7H2O	0.3	N/A	N/A
NaCl	0.43	N/A	N/A
Copper sulfate	N/A	$4.005\ 10^{-5}$	N/A
Sodium selenite	N/A	$1.012\ 10^{-8}$	N/A
Thiamine HCl (vit. B1)	N/A	$2.965\ 10^{-4}$	N/A
Biotin (vit. H)	N/A	$2.049\ 10^{-6}$	N/A

585 N/A: not applicable.
586
587
588

Table 2. Listing of the strains of microalgae tested for methane potential

Strains	Type	Media	TS[a] (g/kg)	TVS[b] (g/kg)
Neochloris oleoabundans UTEX1185	Freshwater	Bold's 3N	225 ± 16	189 ± 14
Chlorella vulgaris UTEX265	Freshwater	Bold's 3N	215 ± 5	200 ± 5
Scenedesmus sp.-PN$_2$	Freshwater	Bold's 3N	292 ± 11	234 ± 1
Chlorella sorokiniana	Freshwater	Bold's 3N	293	255
Chlorella sp. Island-R	Freshwater	Bold's 3N	311	290
Chlamydomonas debaryana-AMB1	Freshwater	Bold's 3N	152	138
Chlamydomonas sp.-AMLS1b	Freshwater	Bold's 3N	163	143
Micractinium sp.-RB1b	Freshwater	Bold's 3N	247	215
Chlorella vulgaris-FGP1	Freshwater	Bold's 3N	296 ± 1	254 ± 5
Isolate SK-RBD8	Freshwater	Bold's 3N	242 ±1	218 ± 1
Isolate SK-RB1a	Freshwater	Bold's 3N	281 ± 1	233 ± 2
Scenedesmus sp.-AMDD Nov-2010	Freshwater	Bold's 3N	242 ± 2	210 ± 1
Scenedesmus dimorphus UTEX1237	Freshwater	f/2	272± 6	246 ± 6
Porphyridium aeruginosa UTEX2618	Freshwater	f/2	201± 8	184 ± 7
Botryococcus braunii UTEX572 Mar-2010	Freshwater	f/2	173	153
Botryococcus braunii UTEX572 Jul-2011	Freshwater	f/2	254 ± 2	240 ± 2
Scenedesmus sp.-AMDD Jul-2011	Freshwater	Wastewater	338 ± 4	330 ± 5
Phaeodactylum tricornutum NCMA1327	Marine	f/2	238 ± 1	205 ± 1
Nannochloropsis gaditana NCMA525	Marine	f/2	287 ± 8	263 ± 9
Thalassiosira weissflogii NCMA1336	Marine	f/2	168 ± 9	133 ± 8
Glossomastix chrysoplasta NCMA1537	Marine	f/2	55	23
Isochrysis spp. NCMA462	Marine	f/2	341 ± 2	305 ± 2

[a] Total solids (TS). Initial TS concentration of the paste collected after centrifugation.

[b] Total volatile solids (TVS). Initial TVS concentration as for TS.

Table 3. Final results from the methane potential assays for all tested microalgae strains

Strains	pH	VSS[a] (g/L)	sCOD[b] (mg/L)	VFA[c, d] (mg/L)	NH_4 (mg/L)	Methane production ($mL/gTVS_{in}$)
Neochloris oleoabundans	7.15 ± 0.04	22.0 ± 1.9	931 ± 172	0	826[d]	308 ± 1
Chlorella vulgaris	7.52 ± 0.16	19.5 ± 0.9	1245 ± 270	0	1052[d]	361 ± 11
Scenedesmus sp.-PN$_2$	7.36 ± 0.11	24.8 ± 0.8	641 ± 13	0	820 ± 19	258 ± 7
Chlorella sorokiniana	7.28	18.2 ± 3.0	839 ± 43	0	788 ± 16	283 ± 4
Chlorella sp. Island-R	7.44	18.6 ± 1.3	686 ± 105	0	863 ± 29	302 ± 9
Chlamydomonas debaryana-AMB1	7.33	19.4 ± 0.7	1839 ± 144	0	943 ± 25	302 ± 11
Chlamydomonas sp.-AMLS1b	7.31	16.0 ± 2.2	1971 ± 59	0	1031 ± 53	333 ± 9
Micractinium sp.-RB1b	7.31	21.3 ± 0.0	1044 ± 47	0	973 ± 42	360 ± 54
Chlorella vulgaris-FGP1	7.44 ± 0.02	25.9 ± 0.6	614 ± 17	0	853 ± 7	263 ± 3
Chlorella sorokiniana-RBD8	7.50 ± 0.01	22.4 ± 5.4	609 ± 30	0	1055 ± 20	331 ± 8
Chlorella sp.-RB1a	7.42 ± 0.04	25.1 ± 2.1	631 ± 13	0	983 ± 8	309 ± 19
Scenedesmus sp.-AMDD Nov-2010	7.35 ± 0.03	21.0 ± 1.0	518 ± 30	0	992 ± 59	306 ± 14
Scenedesmus dimorphus	7.12 ± 0.01	22.3 ± 0.8	643 ± 74	0	761[d]	397 ± 10
Phorphyridium aeruginosa	7.22 ± 0.00	17.0 ± 1.4	N/A	N/A	N/A	352 ± 3
Botryococcus braunii Mar-2010	7.05 ± 0.02	18.3 ± 3.1	2428 ± 461	45	919[d]	343 ± 23
Botryococcus braunii Jul-2011	7.44 ± 0.04	23.5 ± 1.4	847 ± 44	0	824 ± 8	370 ± 9
Scenedesmus sp.-AMDD Jul-2011	7.43 ± 0.08	20.4 ± 1.8	908 ± 82	0	765 ± 8	410 ± 6
Phaeodactylum tricornutum	7.25 ± 0.01	22.1 ± 1.3	1976 ± 167	0	974[d]	362 ± 5
Nannochloropsis gaditana	7.08 ± 0.08	24.4 ± 3.8	518 ± 105	0	716[d]	228 ± 4
Thalassiosira weissflogii	7.30 ± 0.04	25.3 ± 1.6	2768 ± 133	0	1019[d]	265± 15
Glossomastix chrysoplasta	6.98 ± 0.03	21.7 ± 3.5	3675 ± 91	63	495[d]	227 ± 8
Isochrysis spp.	7.66 ± 0.05	19.1 ± 2.1	3505 ± 487	0	1622 ± 105	408 ± 4

[a] VSS: volatile suspended solids
[b] sCOD: soluble chemical oxygen demand
[c] VFA: volatile fatty acid
[d] Values were obtained from pooled aliquots from the triplicate of bottles.

Table 4. Statistical analysis to compare the physico-chemical parameters of the freshwater and marine microalgae at the end of the methane production assays

Parameters	Variance analysis	Result	t-test two samples with equal / unequal variance	Difference between the average values
pH	P = 0.024	Unequal	P = 0.270	Not significant
VSS	P = 0.492	Equal	P = 0.151	Not significant
sCOD	P = 0.008	Unequal	P = 0.035	Significant
Ammonium	P < 0.001	Unequal	P = 0.381	Not significant

alpha: 0.05

1

Table 5. Statistical analysis to compare the methane production of freshwater and marine microalgae grown in Bold's 3N or f/2 media

Parameters	Variance analysis	Result	t-test two samples with equal / unequal variance	Difference between the average values
Freshwater Bold's 3N vs f/2	P = 0.341	Equal	P = 0.004	Significant
Freshwater Bold's 3N vs marine f/2	P = 0.069	Equal	P = 0.348	Not significant
Freshwater f/2 vs marine f/2	P = 0.099	Equal	P = 0.036	Significant

alpha: 0.05

1

Screening microalgae strains for their productivity in methane following anaerobic digestion

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ABSTRACT

Interest in the use of microalgae for the production of biofuels has grown in recent years. Biomethane is a biofuel that can be obtained with high efficiency from anaerobic digestion of various organic feedstocks. In this study, a selection of freshwater (n=15) and marine (n=5) microalgae were tested in order to identify a microalgal strain that could be used as a model for large scale production of methane. Analysis of pH, volatile suspended solids and ammonium at the end of the assay ranged between 6.98- 7.66, 16.0- 25.9 g/L and 495- 1622 mg/L respectively. No significant differences in these values were detected between freshwater and marine strains. There was no significant difference in the methane yield from freshwater microalgae (329 ± 43 $mLCH_4$/gTVS) and marine microalgae (298 ± 83 $mLCH_4$/gTVS) although it varied greatly within the tested strains. A statistical analysis of the microalgae grown under two different culture media showed that the type of medium was more determinant than the type of microalgae (freshwater or marine) for the methane yield, with 310 ± 35, 365 ± 25 and 303 ± 77 $mLCH_4$/gTVS for the freshwater microalgae grown in Bold's-3NV, f/2 and marine microalgae grown in f/2 media, respectively. The strains *Scenedesmus sp.*-AMDD, *Isochrysis* sp. and *Scenedesmus dimorphus* displayed the best methane yield with 410 ± 6, 408 ± 4 and 397 ± 10 $mLCH_4$/gVS, respectively. The strain *Scenedesmus* sp.-AMDD was chosen as a model strain for future work development with continuously fed digesters.

KEYWORDS

anaerobic digestion; methane; microalgae; biofuel; bioenergy; Scenedesmus

1. INTRODUCTION

There is a growing interest in the use of microalgae for the production of biofuels in recent years [1], as algal biomass offers several potential advantages compared with other feedstocks, including higher areal biomass productivity, high lipid content and higher value products [2]. Although past efforts were mainly engaged in the development and processing of microalgae strains for the production of biodiesel [3, 4], conversion of algal biomass into biomethane is drawing increasing attention [5, 6]. The use of the whole microalgae for methane production as a biofuel has been suggested and verified in a life cycle analysis (LCA) [7], which showed that methane compares favourably with other biofuel production scenario. Although it is not yet clear what the most effective process for biofuel production from microalgae is, anaerobic digestion and methane production is certainly the least complex one [5]. Some authors are more assertive, and suggest that the production of methane via anaerobic digestion (AD) is the most feasible and cost-effective route to an energy product [8]. This is supported by Harun et al [9] who demonstrated that more energy could be generated from the production of methane from microalgae (14.04 MJ/kg), rather than biodiesel (6.6 MJ/kg) or ethanol (1.79 MJ/kg) where their unit "kg" is assumed to be "kg of dry weight algae". Furthermore, up to 65% of the chemical energy stored in the algal biomass can be potentially recovered through AD to methane [10].

Anaerobic digestion is already successfully applied to the conversion of a wide variety of organic substrates to methane, such as the organic fraction of municipal solid wastes [11], waste activated sludge [12], and energy crops [13]. Recent studies are increasing our knowledge about anaerobic digestion of microalgae. Theoretical calculations [14] as well as bottle and digester experiments [15] have shown the great potential of anaerobically digesting microalgae for methane production which can be further converted into a clean and renewable biofuel.

Microalgae macromolecular distribution and cell walls renders anaerobic digestion efficiency strain –specific [5]. This was emphasized by Mussgnug et al. [15] who suggested testing strains individually since their methane potential could not be inferred

from their phylogenetic classification. Biomethane potential assays were performed on microalgal biomass, and showed a wide spectrum of methane yields. For example, Zamalloa et al. [16] showed 0.36 and 0.24 L CH_4 g^{-1} volatile solids (VS) for *Phaeodactylum tricornutum* and *Scenedesmus obliquus,* respectively. A conversion efficiency of 51% was obtained from *P. tricornutum* in a continuous digestion in a hybrid flow-through reactor. A similar performance was observed during the digestion of *Chlorella vulgaris* in a 1L digester at 24d HRT, where 51% inlet COD degradation for 240 mL CH_4/g volatile suspended solid (VSS) added was obtained [17]. Fed-batch assays confirmed that the limiting step for algae digestion was the hydrolysis. Recent studies performed in an anaerobic membrane bioreactor with *P. tricornutum* have confirmed that around 50% of the tested microalgal biomass was not degraded into methane [18], thus emphasizing the interest in identifying a potential strain more easily hydrolyzed thus yielding more methane per kg VS, i.e. a higher biofuel production per kg of initial substrate. Alternatively, high lipid content would theoretically improve the methane potential of whole microalgae. However, the cultivation parameters involved (high light intensity, nutrient starvation for example) which would increase the accumulation of lipid in the cells, would come at the expense of microalgae biomass productivity. It is not clear if this particular cultivation mode would result in a higher methane yield and an optimal scenario for microalgae biomass and lipid productivity has still to be determined.

Fermentation of marine microalgae could be inhibited because of high levels of sodium [14]. However, it seems that marine algae are more prone to disintegration when mixed with anaerobic fermenter sludge [15] resulting in the release of more intracellular material which could theoretically enhance methane production. It is not clear which species of freshwater or marine microalgae would be best suited for optimal methane production.

Although there have been recent developments in the field of biomethane production from microalgae, there is still a need to screen multiple strains to identify one that could combine as many of the desired traits as possible: ease of cultivation, high biomass

yields, high protein and/or lipid content and ease of anaerobic biodegradation. The purpose of this study was thus to evaluate the methane potential from a selection of freshwater and marine microalgae grown on two culture media. The final objective was to identify a microalgal strain that could be used as a model for future work and upscaled experiments for biomethane production.

2. MATERIALS AND METHODS

2.1 Growth and culture conditions

The freshwater strains *Neochloris oleoabundans*, *Chlorella vulgaris*, *Scenedesmus dimorphus*, *Porphyridium aerugineum* and *Botrycoccus braunii* were obtained from the University of Texas Culture Collection (strain ids 1185, 265, 1237, 2618 and 572, respectively). The other freshwater strains used in this study but not obtained from the UTEX collection were isolated from the Canadian province of Saskatchewan as described in Park et al. [8]. Some of these strains including *Scenedesmus sp*.-AMDD, *Scenedesmus sp*.-PN2, *Chlamydomonas debaryana*-AMB1, *Chlamydomonas sp*.-AMLS1b, *Chlorella sorokiniana*, *Chlorella sp*. Island-R, *Chlorella vulgaris* and *Micractinium sp*.-RB1b were isolated from soil samples. All of these isolates were photoautotrophically cultivated in Bold's-3NV (B3NV) medium as shown in Table 1 [8]. The marine strains *Phaeodactylum tircornutum*, *Nannochloropsis gaditana*, *Thalassiosira weisflogii*, *Glossomastix chrysoplasta* and *Isochyrsis* spp. (strain ids 1327, 525, 1336, 1537, and 462, respectively) were obtained from the National Centre for Marine Algae and Microbiota (formerly the Provasoli-Guillard Culture Collection of Marine Protozoa), East Boothby, Maine. All marine strains were cultivated in Pasteurized seawater in f/2 media [19] as detailed in Table 1. Table 2 shows the different strains tested for their methane potential along with the specific medium in which they were cultivated and their total solids (TS) content after harvesting by centrifugation and total volatile solids (VS) after combustion. The microalgal biomass was collected by centrifugation (CEPA Z101 process centrifuge; 15,000 x g) at a processing rate of 20 L/min for a typical duration of 30 minutes. Table 2 also lists the strains as either freshwater or marine.

2.2 Preparation of the methane potential assays

Biomass samples of the microalgae strains listed in Table 2 were received and tested between September 2009 and November 2011. The methane potential assays were prepared based on the Biochemical Methane Potential (BMP) assay for wastewater [20]. A few modifications were made to adapt the test to high solid samples [21]. The assays were performed using an inoculum to microalgae ratio of 2:1, based on the VS concentration, to ensure better kinetic constants [22]. The inoculum consisted of 20 g of granular biomass (wet weight) collected from a full scale upflow anaerobic sludge blanket (UASB) digester treating apple processing wastewater (Lassonde Inc., Rougemont, QC, Canada; 45°25'52.71" N, 73°03'12.15" W), with a moisture content of 90%. The inoculum was starved for 48 hours prior to the start-up of the assays, by incubation at 35°C and at agitation at 150 rpm with no substrate. Altough the assays were performed at different times over the two year period of this study, the methanogenic activity of the inoculum was maintained over time.

Triplicate bottles (500 mL) were prepared anaerobically under a constant flow of a gas mix (80% N_2, 20% CO_2) for each experimental digestion. Before sealing, the pH was adjusted to 7.0, if necessary, when the bottles were ready. A typical bottle contained one gVS of the tested microalgae, 2 gVS of inoculum, two mL of defined media, two ml of bicarbonate buffer and 0.5 ml of 1.25% Na_2S-cysteine solution. The recipes for the different solutions and the procedure for their preparation are detailed elsewhere [21]. The final volume was adjusted to 100 mL for all bottles using boiled demineralized water. The bottles were incubated at 35°C with an agitation of 150 rpm. Control bottles were prepared to correct for endogenous methane production from the assays. The control bottles were identical to the test bottles, excepted that the microalgal suspension was replaced with the same volume of deoxygenated water. The assays were conducted until the methane production became negligible (< 3 ml d^{-1}) which typically occurred between 34 and 50 days of incubation.

2.3 Analytical methods

158 The biogas production was released from the bottles at regular interval, generally four
159 times in the first week of incubation, and twice weekly afterward, using a water-
160 displacement system built from a volumetric glass burette, graduated every 0.2 ml. The
161 bottles were allowed to equilibrate by displacing water from the burette to a connected
162 Erlenmeyer flask, which required around 20 seconds to perform. A gas sample (0.3 ml)
163 was then taken from the headspace of the bottles using a model 1750 gas-tight syringe
164 (Hamilton, Reno, USA) and analyzed for H_2, N_2, CH_4 and CO_2 by gas chromatography
165 (GC) as described in Frigon et al [21]. All gas or methane volumes presented in this study
166 are described at standard temperature and pressure, of 273.15 K and 100 kPa pressure.
167
168 A few parameters were monitored on the algae paste and at the end of the incubation for
169 each set of assays, including total solids (TS), total volatile solids (VS), volatile
170 suspended solids (VSS), pH, soluble chemical oxygen demand (sCOD), ammonium
171 (NH_4) and volatile fatty acids (VFA), namely acetate, propionate and butyrate. The pH
172 was measured on an Accumet AP61 portable pH meter equipped with a micro probe
173 (Fisher, Fairlawn, USA) directly on the recovered sample, within one minute of
174 sampling. The TS, VS, VSS and sCOD concentration were determined according to
175 Standard Methods [23] using methods 2540B, 2540D, 2540E and 5220D, respectively.
176 The ammonium and VFA were analyzed by GC [21]. The ammonium concentration was
177 expressed as mg NH_4/L throughout the manuscript.
178
179 ***2.4 Statistical analysis***
180 As a first step, the homogeneity of variance was tested using Levene's F-test [24]. This
181 test provides a significance value (P-value). If P is greater than the significance level of
182 0.05 (alpha), the group variances can be treated as equal. Otherwise ($P < 0.05$), we have
183 unequal variances. Then a Student's t-test was performed to determine whether there was
184 a statistically significant difference between the means in the two groups when variances
185 were equal. Otherwise, a Welch's t-test was used [25]. In both t-tests, the means from the
186 two unrelated groups were considered as not significantly different (null hypothesis)
187 when the P-value was greater than the significance level of 0.05 (alpha). All statistical

tests were performed using Microsoft Excel (Microsoft Corporation, Redmond, Washington).

3. RESULTS AND DISCUSSION

3.1 Results for the physico-chemical parameters for all methane potential assays

Methane potential assays were performed in triplicate for 15 freshwater and 5 marine microalgae strains. Table 3 presents the results obtained at the end of the incubation period for all tested strains. The pH was measured at the end of each assay and varied between 6.98 ± 0.03 and 7.66 ± 0.05. In parallel to a neutral pH, there was no VFA accumulation at the end of the incubation period for almost all of the assays reported. The VFA concentration was low for the two strains with reported VFA, *B. braunii* Mar-2010 and *G. chrysoplasta*. The neutral pH and the absence of VFA accumulation are thus indications that no irreversible inhibition occurred and conditions were satisfactory by the end of the test.

Table 3 presents results for the volatile suspended solids (VSS), sCOD and VFA concentration obtained at the end of the methane potential assays. Although it can be presumed that a high level of microalgae degradation in the assays would result in lower VSS concentration at the end of the incubation, there were no strong correlation between the final VSS concentrations in the assays and the methane production for either the freshwater (coefficient of correlation (R = 0.322) and marine (R = 0.535) microalgae. This could be related to the high amount of inoculum which contributed to 2/3 of the initial VSS content in the bottles. The final soluble COD concentration can give an indication of the amount of substrate hydrolyzed but recalcitrant to further mineralization toVFA and then methane and CO_2. The sCOD concentration were low in general for the freshwater strains but rather high for the marine strains. It can be presumed that the high sCOD concentration represented recalcitrant or non biodegradable material.

The average final ammonium (NH_4) concentration was 351 ± 16 mg/L in the control bottles containing only inoculum. Not including *Isochrysis sp.*, an average final

ammonium concentrations of 883 ± 140 mg/L was observed for all tested strains. The final ammonium concentration in digestions containing *Isochyrsis sp.* was 1622 ± 105 mg/L, which was considered an outlier. These concentrations were well below those considered inhibitory [26]. These values are further indications of the proper conditions in the assays, and shows that the digestion of the microalgae at low initial VSS concentration would greatly reduces the potential of ammonia for feedback inhibition on methanogenesis as reported by Heaven et al. [27].

The variances of the average pH, VSS, sCOD and ammonium concentrations for the freshwater and marine microalgae were compared, followed with a t-test, in order to determine whether the means of the physico-chemical parameters were significantly different for the two groups of microalgae (Table 4). The resulting P values were 0.270, 0.151, 0.035 and 0.381 for pH, VSS, sCOD and ammonium, respectively. Therefore, there were no significant differences between the final pH, VSS and ammonium concentration between the freshwater and marine microalgae at the end of the digestion assays. However, the sCOD concentration were significantly higher in the case of the marine microalgae, except for *N. gaditana*.

3.2 Overview of the methane production for all assays

The methane production for all tested strainsvaried between 227 -410 mL CH_4/gTVS. Representative time-courses showing the kinetics of methane accumulation from digestion of five microalgae strains over time are shown in Figure 1. The onset of methane production appeared to take place without delay in the assays, probably due to the fast initial transformation of soluble biodegradable matter. Initial methane production (day 0 – 3) was significantly different for the tested strains and reached 19, 23.5, 23.3, 23.8 and 37.5 mL $CH_4/gVSS_{inoculum}$.d for *C. sorokiniana*, *Chlorella sp. Island-R*, *C. debaryana-AMB1*, *C. sp.-AMLS1b* and *Micractinium sp.*, respectively. While *Micractinium sp.* displayed the highest initial methane production for all tested strains, *Thalassiosira weisflogii* showed the lowest methane production at 8.3 mL $CH_4/gVSS_{inoculum}$.d. This could be due to the presence of the silica frustules which might

have impeded digestibility. *Micractinium sp.*, which showed the highest initial methane production, also yielded more methane than the other strains from Figure 1.

A decrease in the methane production kinetic was observed after the first four days in almost all of the anaerobic digestion assays performed in this study, and persisted until days 14 to 17 of incubation. This latency could be related to high lipid content and partial inhibition from long chain fatty acids (LCFA) [28]. However, the inflection in the methane production kinetic could be caused more simply by a physical barrier such as potentially recalcitrant algal cells impeding hydrolysis and preventing the release of soluble biodegradable compounds. There was no such inflection in the methane production for *Micractinium sp.* (Figure 1), as well as in *Scenedesmus dimorphus*, *Isochrysis sp.*, *Glossomastix chrysoplasta* and SK-RB1a (data not shown). *Isochrysis sp and Scenedesmus dimorphus* had the 2nd and 3rd highest final methane yields out of 20 strains tested.

It is possible to estimate a theoretical methane yield from microalgae biomass based on an average elemental formula of $C_{2.11}H_{3.93}ON_{0.26}$ [27]. The maximal CH_4 yield would then be 0.55 Nm^3 CH_4/kgTVS, although this can probably not be achieved in practice due to recalcitrant material that is always present in any organic matter. This generic stochiometric value could also underestimate the methane yield achievable from lipid-rich microalgae. For instance, microalgae containing 40% lipids, 20% carbohydrates and 28% proteins would have a theoretical yield as high as 0.68 Nm^3 CH_4/kgTVS. This highlights the importance of using actual assayed values of methane production from algal biomass rather than theoretical estimates. The final methane production from *Scenedesmus* sp.AMDD-Jul 2011 and *Isochrysis spp.* represented between 60 and 75 % of the theoretical methane yields predicted from the high-lipid and average elemental stochiometries mentioned above, respectively.. The relatively high methane yields obtained from *Scenedesmus sp.*-AMDD and *Isochrysis sp.* indicates they may be good candidates for large-scale production.

Previous studies have discussed the similarities and differences of microalgal biomass and waste activated sludge (WAS) regarding their composition and anaerobic

280 degradation potential [17, 29]. In this study, it was shown that methane production from
281 microalgae was a relatively fast process, with digestion times that were comparable to
282 what is required for municipal sludge (20-40 days) [30]. The methane yield was over 330
283 Nm^3 CH_4/kgTVS for 50% of the microalgae strains tested (Table 1), representing a
284 conversion efficicency of 60% using the stochiometric formula detailed above, and this
285 would suggest better amenability to biodegradation than WAS [12].
286
287 One factor that could have contributed to the high methane yields obtained in this study
288 could be the freezing of the microalgae paste for storage prior to shipment between
289 collaborating laboratories. This can be considered a form of pretreatment that may to
290 some extent disintegrate the microalgae prior to digestion. Freeze thaw cycling is known
291 to cause a decrease in the volatile solids (TVS) of mixed sewage sludge simultaneously
292 with an increase of the soluble COD and VFAs, thereby improving biogas yield [31].
293 This is consistent with the results of Harith et al [32], who showed that freezing the
294 marine diatom *Chaetoceros calcitrans* at -20°C for 2 weeks decreased its viability upon
295 thawing. Another positive aspect of the present study is our use of wet algal biomass. The
296 use of dried algae biomass has been shown to reduceits digestibility compared to wet
297 material [33].
298
299 Some of the microalgal strains tested in this study have been reported to contain high oil
300 content (% dry wt): *Botryococcus braunii* (25-75), *Chlorella* (28-32), *Isochrysis sp.* (25-
301 33), *Nannochloropsis sp.* (31-68), *Neochloris oleoabundans* (35-54), *Phaeodactylum*
302 *tricornutum* (20-30) (extracted from Table 2 in [4]). Their methane production ranged
303 from very low (228 mL CH_4/gTVS for *Nannochloropsis gaditana*) to high (408 mL
304 CH_4/gTVS for *Isochrysis sp.*, Table 3). The low yield obtained for *Nannochloropsis*
305 could be related to its tough cell wall, caused by the presence of sporopollenin polymers
306 [34]. The high methane production from the digestion of *B. braunii* (343-370 mL
307 CH_4/gTVS) could be due to the presence of an external lipid biofilm matrix thatholds the
308 fan-shaped colonies of *B. braunii* together [35]. Six different strains of *Chlorella* were
309 tested in this screening study and their methane yields were lower than in previous
310 reports, except for *C. vulgaris* at 361 ± 11 mL CH_4/gTVS, possibly due to their

311 recalcitrant cellulosic cell walls [36]. Among the strains listed above, *Isochrysis sp*.
312 showed the highest methane production (408 mL CH_4/gTVS). *Isochrysis sp*. is known to
313 synthesize high amounts of lipids, mainly polyunsaturated fatty acids (PUFAs) [37].
314 Furthermore, the absence of a tough cell wall makes this strain an interesting prospect for
315 biofuel production.
316
317 The highest methane yield (410 ± 6 mL/gTVS_{in}) was obtained with wastewater-grown
318 *Scenedesmus* sp.-AMDD, despite previous reports that *Scenedesmus* are supposed to be
319 highly recalcitrant to digestion due to a tough polysaccharide-based cell wall [15, 38].
320 This is in contrast to the findings of Mussgnug *et al* [15] where a relatively low methane
321 yield of 287 mL/gVS was reported from *Scenedesmus obliquus*. Light microscopy photos
322 even showed intact cells after prolonged anaerobic incubation, and their hypothesis for
323 methane production within the digester included methane from debris transferred with the
324 culture or biodegradable metabolites provided by the activity of *Scenedesmus* within the
325 digester. Presumably, the specific inoculum used in our BMP assays had a stronger
326 cytolytic activity than inocula from other studies. A higher cellulase activity in the assay
327 would favor the disruption of the cell wall and membrane of the microalgae [39], thus
328 allowing a higher methane production.
329
330 However, the *Scenedesmus sp.*-AMDD strain yielded significantly less methane (306 ±
331 14 mL CH_4/gTVS) when growing in the Bold's 3N medium as compared with
332 wastewater. In a related study, an average methane production yield of 340 mL/gTVS
333 (for a 56% conversion efficiency) was obtained with *Scenedesmus sp.*-AMDD grown on
334 a different municipal wastewater [10]. The difference observed for the three experiments
335 with *S. sp*-AMDD supports the view that factors such as the culture medium and growth
336 conditions could have a significant impact on the specific methane yield. Methane yields
337 from digestions of specific algae strains grown in the same medium are generally less
338 variable than when grown in different media. For instance, the methane production from
339 two *Botryococcus braunii* assays grown in f/2 medium fifteen months apart reached 342
340 ± 23 and 370 ± 10 mL CH_4/gTVS, respectively.
341

342 3.3. Comparison of the methane production results from the freshwater and marine strains
343 One of the objectives of this study was to compare the methane production potentials
344 obtained from freshwater versus marine microalgae. It is interesting to note that both
345 freshwater (*Scenedesmus sp.*-AMDD, 410 mL CH_4/gTVS) and marine (*Isochrysis sp.*,
346 408 mL CH_4/gTVS) microalgae have the potential to generate high yields of methane
347 after anaerobic digestion. Figure 2 presents the methane produced for all screened strains,
348 grouped between freshwater and marine microalgae. The average methane production
349 from the freshwater microalgae was 329 ± 43 mL CH_4/gTVS, compared with 298 ± 83
350 mL CH_4/gTVS for the marine strains. It can be clearly seen from the size of the boxes
351 and the standard deviations, that the methane production varied greatly, in particular for
352 the marine strains. The data from both groups were processed through an F-test resulting
353 in unequal variances (P = 0.027), followed by a t-test showing no significant difference
354 (P = 0.229) between the methane yields obtained from freshwater or marine microalgae.
355 The choice of a microalgal strain for methane production will therefore have to be made
356 considering the different aspects of the culture of the model strain (productivity, use of
357 land, harvesting).
358
359 3.4. Comparison of the methane production results as a function of the cultivation
360 medium
361 All the marine strains tested in this study, along with four freshwater strains, were grown
362 on f/2 medium. Figure 3 shows the methane production results, grouped with respect to
363 the growth medium, and with a further separation between B3NV and f/2 media for the
364 freshwater strains. The average methane production from the freshwater microalgae was
365 310 ± 35 and 365 ± 25 mL CH_4/gTVS with B3NV and f/2 media, respectively. The
366 average methane production for the marine microalgae grown on f/2 medium reached 298
367 ± 83 mL CH_4/gTVS. As mentioned in Section 3.3, the methane production seemed to
368 vary more for the marine strains.
369
370 The three groups of data were processed through an F-test for variance, followed by a t-
371 test assuming equal / unequal variances to evaluate if their means were equal or
372 statistically different, as reported in Table 5. The statistical analysis was performed using

373 the average values from the triplicates, i.e. performed on 12, 4 and 5 values for the B3NV
374 and f/2 media for freshwater microalgae and f/2 medium for marine microalgae,
375 respectively. There was a significant difference (P = 0.004) in the methane production
376 results for the freshwater strains between B3NV and f/2 media. A comparison between
377 B3NV and f/2 medium revealed that the B3NV medium is significantly richer in nutrients
378 with 10 times more nitrates and 47 times more phosphates (Table 1). The f/2 medium
379 could have promoted the accumulation of lipids in the algae strains which would have
380 resulted in higher methane production after anaerobic digestion (Figure 3). B3NV
381 medium also contained much more cobalamin (vitamin B12). However, the exact role of
382 cobalamin in the microalgae metabolism is still unknown and around half of the
383 microalgae species can synthetize their own cobalamin [40]. Therefore the potential
384 benefits of a higher cobalamin dose could not be confirmed as the capacity of each of the
385 tested micro-algae for B_{12} synthesis is unknown.
386
387 There was also a significant difference (P = 0.036) in the methane production results for
388 the freshwater and marine strains grown on f/2 media, although the low number of
389 samples from which the means were obtained could limit the statistical significance of
390 the test.
391
392 3.5. Cost aspects of producing methane from microalgal biomass
393 A recent cost analysis [41] concluded that methane production and cogeneration from
394 microalgal biomass would become profitable from a feed-in tariff (FIT) of €0.133/kWh
395 for both heat and electricity on an equal basis and a carbon credit of €30/t eCO_2, although
396 the latter would only represent 4% of the revenue. The analysis assumed that the algal
397 culture in raceway ponds can have a minimal productivity of 90 dry t/ha.yr, be
398 concentrated up to 20–60 dry kg/m^3 at the harvest, which is estimated to represent a
399 feedstock cost of €86–€124/dry t, and that the algal concentrate can be processed in an
400 anaerobic reactor at a loading rate of 20 kg VS/m^3·d with a conversion efficiency of 75%.
401 Our results show that a number of microalgal strains have a methane potential near or
402 above 0.4 Nm^3/kg VS (i.e. corresponding to a conversion efficiency of ca. 75%), which
403 would match or even lower the minimum FIT for profitability in the above case study. A

variety of pre-treatment techniques could certainly improve the methane production from microalgal biomass, and accordingly increase the revenue [6]. But the addition of a pre-treatment stage would also increase the capital and operation costs, which may not be offset by the gain in methane.

4. CONCLUSIONS

The identification of a particular microalgae strain as a model for biofuel production represents a challenge considering that many parameters such as high biomass and lipid yields, which are often mutually exclusive, have to be taken into account. The approach that was favored in this study was to target strains with a high dry weight to culture volume ratio.

In this study, a screening of the methane production potential of freshwater and marine microalgae was performed in order to identify the most promising strain for further work development. Specifically, the highest methane production was obtained from *Scenedesmus dimorphus*, *Scenedesmus sp.* AMDD and *Isochrysis sp*., among the 20 tested strains. Some interesting outcomes were derived from these assays, such as the demonstration that high methane production can be obtained from previously reported hard to digest microalgae strains, without any preliminary pretreatment aside from the potential impact of freezing / thawing, with unadapted anaerobic inoculum. Also, the impact of the growth medium on the resulting methane production from the microalgae was shown to be significant, independant of the type of water in which the microalgae are grown.

Among the three highest methane yielding strains, *Scenedesmus sp.* AMDD was chosen for further study, for practical reasons, as it is robust, easy to cultivate and generates high biomass yields on municipal wastewater. Future work will include continuous digestion of microalgal biomass in lab-scale digesters, and the use of thermal and chemical pretreatments in order to increase the methane production.

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Figure captions

Figure 1. Typical time courses of methane production from anaerobic digestion of five microalgae strains. The cumulative methane production for each of the strain is expressed in mL of methane produced per gram of total volatile solids of microalgae added in the test bottles. The methane production shown is a net production, e.g. with endogenous control removed.

Figure 2. Comparison of the amount methane produced from freshwater versus marine microalgae strains. The methane production for each category of microalgae is expressed in mL of methane produced per gram of total volatile solids of microalgae added in the test bottles. The box plot can be described as follow: the lower and upper limit of the box represents the lower (25%) and upper quartile (75%) for the data distribution. In other words, 50% of the methane production values are comprised within the box. The line inside the box represents the median value (50%). The whiskers represent the minimum and the maximum values for each category of microalgae.

Figure 3. Comparison of the amount methane produced from the microalgae strains as a function of the culture growth medium. The methane production for each category of microalgae is expressed in mL of methane produced per gram of total volatile solids of microalgae added in the test bottles. The box plot can be described as follow: the lower and upper limit of the box represents the lower (25%) and upper quartile (75%) for the data distribution. In other words, 50% of the methane production values are comprised within the box. The line inside the box represents the median value (50%). The whiskers represent the minimum and the maximum values for each category of microalgae.

583 Table 1. Comparison between the composition of the Bold's 3N and f/2 media
584

Compound	Bold's 3N (mM)	f/2 (mM)	Ratio Bold/f2
NaNO3	8.82	0.882	10
$FeCl_3 \cdot 6H_2O$	$2.16\ 10^{-3}$	$1.202\ 10^{-2}$	0.2
$MnCl_2 \cdot 4H_2O$	$1.26\ 10^{-3}$	$8.843\ 10^{-4}$	0.1
Zinc chloride / sulfate 84%	$2.22\ 10^{-4}$	$7.826\ 10^{-5}$	2.8
$CoCl_2 \cdot 6H_2O$	$5.04\ 10^{-5}$	$4.203\ 10^{-5}$	1.2
$Na_2MoO_4 \cdot 2H_2O$	$1.02\ 10^{-4}$	$3.640\ 10^{-5}$	2.8
$Na_2EDTA \cdot 2H_2O$	$1.02\ 10^{-2}$	$1.142\ 10^{-2}$	1.1
Sodium phosphate	1.72	$3.623\ 10^{-2}$	47
Vitamin B12	$1.0\ 10^{-4}$	$3.687\ 10^{-7}$	271
CaCL2.2H2O	0.17	N/A	N/A
MgSO4.7H2O	0.3	N/A	N/A
NaCl	0.43	N/A	N/A
Copper sulfate	N/A	$4.005\ 10^{-5}$	N/A
Sodium selenite	N/A	$1.012\ 10^{-8}$	N/A
Thiamine HCl (vit. B1)	N/A	$2.965\ 10^{-4}$	N/A
Biotin (vit. H)	N/A	$2.049\ 10^{-6}$	N/A

585 N/A: not applicable.
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588

Table 2. Listing of the strains of microalgae tested for methane potential

Strains	Type	Media	TS[a] (g/kg)	TVS[b] (g/kg)
Neochloris oleoabundans UTEX1185	Freshwater	Bold's 3N	225 ± 16	189 ± 14
Chlorella vulgaris UTEX265	Freshwater	Bold's 3N	215 ± 5	200 ± 5
Scenedesmus sp.-PN$_2$	Freshwater	Bold's 3N	292 ± 11	234 ± 1
Chlorella sorokiniana	Freshwater	Bold's 3N	293	255
Chlorella sp. Island-R	Freshwater	Bold's 3N	311	290
Chlamydomonas debaryana-AMB1	Freshwater	Bold's 3N	152	138
Chlamydomonas sp.-AMLS1b	Freshwater	Bold's 3N	163	143
Micractinium sp.-RB1b	Freshwater	Bold's 3N	247	215
Chlorella vulgaris-FGP1	Freshwater	Bold's 3N	296 ± 1	254 ± 5
Isolate SK-RBD8	Freshwater	Bold's 3N	242 ±1	218 ± 1
Isolate SK-RB1a	Freshwater	Bold's 3N	281 ± 1	233 ± 2
Scenedesmus sp.-AMDD Nov-2010	Freshwater	Bold's 3N	242 ± 2	210 ± 1
Scenedesmus dimorphus UTEX1237	Freshwater	f/2	272± 6	246 ± 6
Porphyridium aeruginosa UTEX2618	Freshwater	f/2	201± 8	184 ± 7
Botryococcus braunii UTEX572 Mar-2010	Freshwater	f/2	173	153
Botryococcus braunii UTEX572 Jul-2011	Freshwater	f/2	254 ± 2	240 ± 2
Scenedesmus sp.-AMDD Jul-2011	Freshwater	Wastewater	338 ± 4	330 ± 5
Phaeodactylum tricornutum NCMA1327	Marine	f/2	238 ± 1	205 ± 1
Nannochloropsis gaditana NCMA525	Marine	f/2	287 ± 8	263 ± 9
Thalassiosira weissflogii NCMA1336	Marine	f/2	168 ± 9	133 ± 8
Glossomastix chrysoplasta NCMA1537	Marine	f/2	55	23
Isochrysis spp. NCMA462	Marine	f/2	341 ± 2	305 ± 2

[a] Total solids (TS). Initial TS concentration of the paste collected after centrifugation.

[b] Total volatile solids (TVS). Initial TVS concentration as for TS.

Table 3. Final results from the methane potential assays for all tested microalgae strains

Strains	pH	VSS[a] (g/L)	sCOD[b] (mg/L)	VFA[c, d] (mg/L)	NH_4 (mg/L)	Methane production ($mL/gTVS_{in}$)
Neochloris oleoabundans	7.15 ± 0.04	22.0 ± 1.9	931 ± 172	0	826[d]	308 ± 1
Chlorella vulgaris	7.52 ± 0.16	19.5 ± 0.9	1245 ± 270	0	1052[d]	361 ± 11
Scenedesmus sp.-PN$_2$	7.36 ± 0.11	24.8 ± 0.8	641 ± 13	0	820 ± 19	258 ± 7
Chlorella sorokiniana	7.28	18.2 ± 3.0	839 ± 43	0	788 ± 16	283 ± 4
Chlorella sp. Island-R	7.44	18.6 ± 1.3	686 ± 105	0	863 ± 29	302 ± 9
Chlamydomonas debaryana-AMB1	7.33	19.4 ± 0.7	1839 ± 144	0	943 ± 25	302 ± 11
Chlamydomonas sp.-AMLS1b	7.31	16.0 ± 2.2	1971 ± 59	0	1031 ± 53	333 ± 9
Micractinium sp.-RB1b	7.31	21.3 ± 0.0	1044 ± 47	0	973 ± 42	360 ± 54
Chlorella vulgaris-FGP1	7.44 ± 0.02	25.9 ± 0.6	614 ± 17	0	853 ± 7	263 ± 3
Chlorella sorokiniana-RBD8	7.50 ± 0.01	22.4 ± 5.4	609 ± 30	0	1055 ± 20	331 ± 8
Chlorella sp.-RB1a	7.42 ± 0.04	25.1 ± 2.1	631 ± 13	0	983 ± 8	309 ± 19
Scenedesmus sp.-AMDD Nov-2010	7.35 ± 0.03	21.0 ± 1.0	518 ± 30	0	992 ± 59	306 ± 14
Scenedesmus dimorphus	7.12 ± 0.01	22.3 ± 0.8	643 ± 74	0	761[d]	397 ± 10
Phorphyridium aeruginosa	7.22 ± 0.00	17.0 ± 1.4	N/A	N/A	N/A	352 ± 3
Botryococcus braunii Mar-2010	7.05 ± 0.02	18.3 ± 3.1	2428 ± 461	45	919[d]	343 ± 23
Botryococcus braunii Jul-2011	7.44 ± 0.04	23.5 ± 1.4	847 ± 44	0	824 ± 8	370 ± 9
Scenedesmus sp.-AMDD Jul-2011	7.43 ± 0.08	20.4 ± 1.8	908 ± 82	0	765 ± 8	410 ± 6
Phaeodactylum tricornutum	7.25 ± 0.01	22.1 ± 1.3	1976 ± 167	0	974[d]	362 ± 5
Nannochloropsis gaditana	7.08 ± 0.08	24.4 ± 3.8	518 ± 105	0	716[d]	228 ± 4
Thalassiosira weissflogii	7.30 ± 0.04	25.3 ± 1.6	2768 ± 133	0	1019[d]	265± 15
Glossomastix chrysoplasta	6.98 ± 0.03	21.7 ± 3.5	3675 ± 91	63	495[d]	227 ± 8
Isochrysis spp.	7.66 ± 0.05	19.1 ± 2.1	3505 ± 487	0	1622 ± 105	408 ± 4

[a] VSS: volatile suspended solids
[b] sCOD: soluble chemical oxygen demand
[c] VFA: volatile fatty acid
[d] Values were obtained from pooled aliquots from the triplicate of bottles.

Table 4. Statistical analysis to compare the physico-chemical parameters of the freshwater and marine microalgae at the end of the methane production assays

Parameters	Variance analysis	Result	t-test two samples with equal / unequal variance	Difference between the average values
pH	P = 0.024	Unequal	P = 0.270	Not significant
VSS	P = 0.492	Equal	P = 0.151	Not significant
sCOD	P = 0.008	Unequal	P = 0.035	Significant
Ammonium	P < 0.001	Unequal	P = 0.381	Not significant

alpha: 0.05

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Table 5. Statistical analysis to compare the methane production of freshwater and marine microalgae grown in Bold's 3N or f/2 media

Parameters	Variance analysis	Result	t-test two samples with equal / unequal variance	Difference between the average values
Freshwater Bold's 3N vs f/2	P = 0.341	Equal	P = 0.004	Significant
Freshwater Bold's 3N vs marine f/2	P = 0.069	Equal	P = 0.348	Not significant
Freshwater f/2 vs marine f/2	P = 0.099	Equal	P = 0.036	Significant

alpha: 0.05

1

Figure 1

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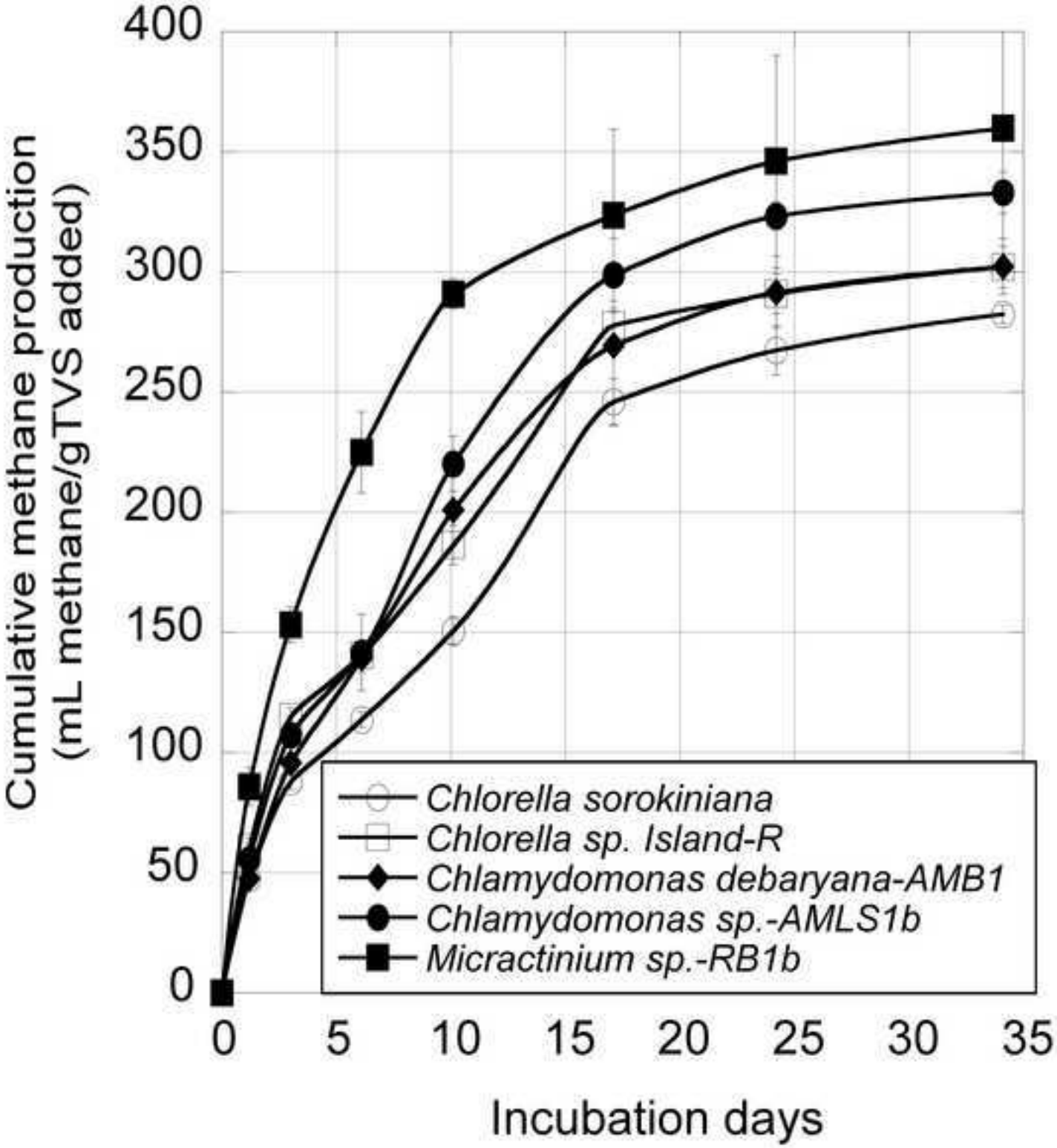

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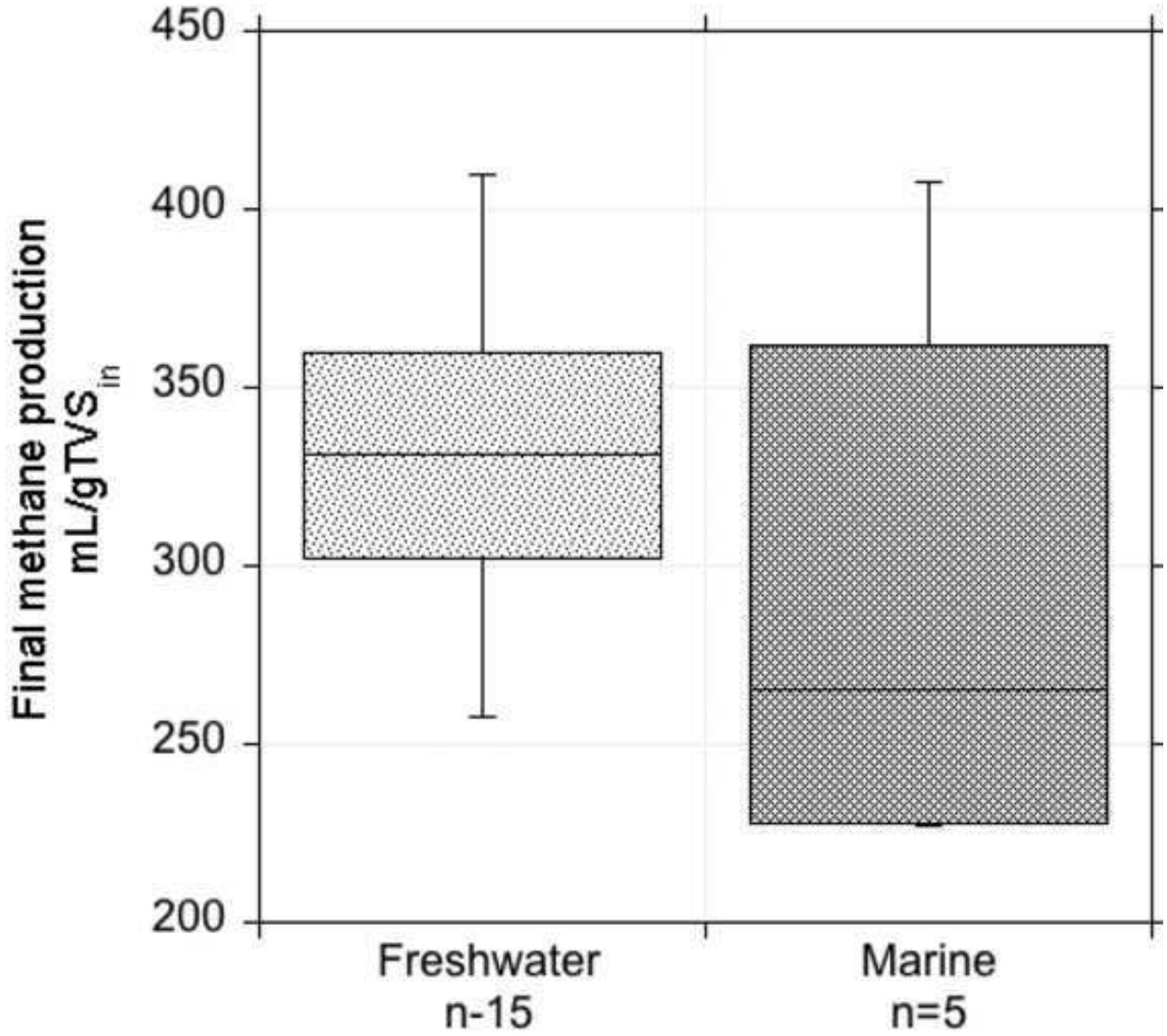

Figure 3
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