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Carbon conversion efficiency and population dynamics of a marine algae–bacteria consortium growing on simplified synthetic digestate: First step in a bioprocess coupling algal production and anaerobic digestion

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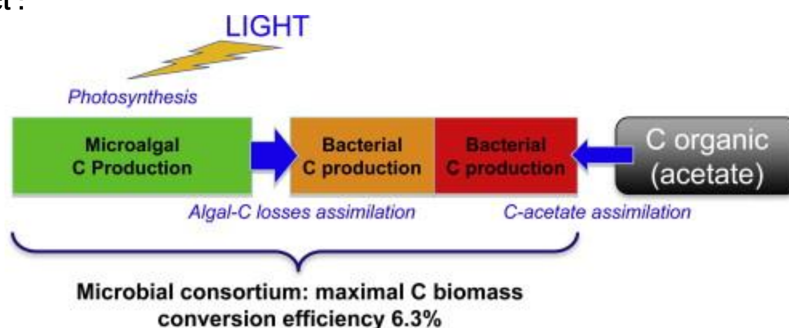
Abstract :

Association of microalgae culture and anaerobic digestion seems a promising technology for sustainable algal biomass and biogas production. The use of digestates for sustaining the growth of microalgae reduces the costs and the environmental impacts associated with the substantial algal nutrient requirements. A natural marine algae–bacteria consortium was selected by growing on a medium containing macro nutrients (ammonia, phosphate and acetate) specific of a digestate, and was submitted to a factorial experimental design with different levels of temperature, light and pH. The microalgal consortium reached a maximum C conversion efficiency (i.e. ratio between carbon content produced and carbon supplied through light photosynthetic C conversion and acetate) of 3.6%. The presence of bacteria increased this maximum C conversion efficiency up to 6.3%. The associated bacterial community was considered beneficial to the total biomass production by recycling the carbon lost during photosynthesis and assimilating organic by-products from anaerobic digestion.

Graphical abstract :



Highlights

► Carbon conversion efficiency of marine microalgae growing on a synthetic anaerobic digestate was 4%. ► Carbon conversion efficiency increased to 6.3% when bacteria were included into the carbon budget. ► Bacteria recycled the carbon lost during photosynthesis and originated from anaerobic digestion.

Keywords : Optimization ; *Nannochloris* ; Heterotrophic bacteria ; Anaerobic digestion ; Bioenergy

1. Introduction

Sustainable production of biogas by coupling microalgal mass production and anaerobic digestion is a promising technology for supplying bioenergy, in terms of production rates (Sialve et al., 2009) and environmental impact (Collet et al., 2011). The advantages of such coupling for large scale energy production are based on the direct use of organic residues (or by-products of biomass extracts/residues) of anaerobic digestion such as volatile fatty acids (*eg.* acetate), phosphorus and nitrogen compounds (phosphate and ammonia) which sustain the growth of photosynthetic biomass and reduces the cost of culture media and final effluent reduction.

Not all algal species can grow efficiently using acetate and ammonia (NH_4^+) as main substrates. A few algal species have shown potential mixotrophy when growing on acetate (Perez-Garcia et al., 2011) while microalgae usually assimilate ammonium more easily than nitrate (Eppley et al 1969). However, the assimilation of the ammonium cation is dependent on pH which may vary considerably especially in non-buffered freshwaters resulting in the ammonia being toxic at high pH (Källqvist & Svenson, 2003). The use of marine waters as culture media appears to be a sustainable alternative for algal mass production freshwaters. Seawater is naturally buffered by carbonates and the salt could be considered to provide an osmotic barrier to the invasive growth of potential competitors.

A marine consortium constituted mainly of *Nannochloris* spp and an associated diverse bacterial community was isolated from a naturally highly-productive temperate marine coastal ecosystem (Thau Lagoon: Western Mediterranean). This consortium resulted from a 7-month batch culture using artificial seawater supplemented with PO_4^{3-}, NH_4^+ and acetate as P, N and C sources, without added vitamins. In this type of consortium, heterotrophic bacteria assimilate acetate naturally for their metabolism and may act as probiotics providing essential compounds (such as vitamins, CO_2) for microalgal growth (Grossart & Simon, 2007). Negative effects of bacteria are however possible when acting as parasites on microalgae leading to the death of their hosts and when efficiently competing for limiting nutrients (Grossart & Simon, 2007 and reference therein). As in natural conditions, the growth of the algal-bacterial consortium and resulting biomass will be driven by physical and chemical factors such as temperature, light and pH, with growth optima being potentially different for bacteria and microalgae. *Nannochloris spp* has been studied since the early 1980s for its potential in large-scale open air culture (Witt et al., 1981). It was grown on a large scale to serve as food for rotifers, copepods, and oysters in aquaculture (Witt et al., 1981). *Nannochloris* spp was found to be dominant in highly eutrophic waters of low salinity as well as in sea water/waste water mixtures. This marine species proved to be euryhaline, eurythermic and insensitive to nutrient changes (Cho et al., 2007). Previous studies have suggested that given its adaptability, rapid growth rate and high nutritional value, *Nannochloris* is an excellent marine primary producer for industrial purposes (Cho et al., 2007). The halotolerant species *Nannochloris oculata* had an optimum temperature of 23-25°C (Cho et al., 2007; Henley et al., 2002). This suggests that growth of *Nannochloris* spp is well adapted to high light and high temperature environments such as those encountered in Mediterranean areas and is also tolerant to variations in pH and salinity (Henley et al., 2002).

Although the culture of freshwater microalgae-bacteria consortia in sewage has been studied since the 50's (Oswald et al., 1953), little is known about the potential of natural

assemblages of marine microbial consortia to produce high biomass when coupled with an anaerobic digester.

A factorial experimental design with different levels of temperature, light and pH in batch culture was performed in order i) to optimize the growth conditions of the algal-bacterial consortium under macronutrients specific of a simplified digestate and ii) to investigate the relative effects of these factors, and their synergetic interactions, on the production of biomass and associated bacterial diversity. The factorial design appeared to allow effective evaluation of limiting factors in phytoplankton communities, saving time and space and providing statistical robustness (Leboulanger et al., 2006).

2. Material and methods

2.1. The marine consortium selected and culture conditions prior to experiments

The natural marine microbial consortium, algae-bacteria, was originally isolated from Thau Lagoon, southern France (43°24’53’’N, 3°41’16’’E). The primary production of this lagoon supports one of the most productive oyster farms in France, as the highly productive microalgal communities are fed by recycled nutrients such as ammonium (Fouilland et al., 2004). Prior to the experiment undertaken in the present work, this consortium was maintained for 7 months at 20°C in batch culture in seawater enriched using a modified Conway medium.

Anaerobic digestion is a biological process wich converts organic matter into biogas and a nutrient rich subproduct, the digestate mineralizing organic matter containing nitrogen and phosphorus. In addition to methane, anaerobic digestion of organic matter produces ammonium and phosphate, which can then be used as a substrate for the microalgae, and volatile fatty acids such as acetate (Sialve et al., 2009). In the present modified Conway medium, besides all trace elements in enrichment media, ammonia (NH_4Cl) replaced nitrate ($NaNO_3$) as the sole source of nitrogen, NaH_2PO_4 was added as source of phosphorus and acetate ($NaC_2H_3O_2$) was added as well. The initial acetate concentration was 92.5 ± 2.1 mg L^{-1}, phosphate concentration was 30 mg L^{-1}, and the ammonia concentration was 47.5 ± 1.9 mg L^{-1}. The nitrogen, phosphorus and carbon concentration used in this study were in the range of concentrations measured in various liquid digestates from previous studies (Albuquerque et al. 2012; Ehimen *et al*, 2011), and using a dilution factor of 10, as in Wang *et al*., (2009) where *Chlorella* was cultivated on an anaerobic digested dairy manure. In the present study, these macronutrients (N and P) are provided in non limiting concentrations, allowing the study of the effect of acetate on the algae-bacteria consortium.

Light was maintained at an average of 261 ± 20 µE m^{-2} s^{-1} using OSRAM L18W/954 daylight fluorescent tubes.

2.2. Experimental design

In order to optimize growth conditions of the microalgae-bacteria consortium, a 2^{3*} central composite design (CCD) was used (Lundstedt et al., 1998). Optimization focused on temperature, irradiance and pH known to influence biomass production significantly. There were 17 photobioreactor (PBR) conditions in a single set of experiments generated around a proposed central point (temperature: 25°C, irradiance: 230 µE m^{-2} s^{-1} and pH: 8). A combination of the 5 levels of the experimental factors was

applied in 14 PBRs and 3 PBRs were replications at the central point allowing for the evaluation of experimental variance. The optimal culture conditions for microalgal, bacterial and total organic content were estimated by statistical analysis using Statgraphics Centurion XV (StatPoint Technologies, Inc., USA).

The experimental equipment used was described in detail in Marchetti et al. (2012). Each of the 17 PBRs was filled with 250 ml of freshly prepared, optically clear microbial consortium culture (initial concentration of microalgae = 7.3 $\times 10^6$ cell $ml^{-1,}$ and optical density at 680 nm of 0.069) limiting self-shading within the cells. During the 10 day batch experiment, photosynthetically available radiation (PAR, 400-700 nm) was continuously supplied through fiber optics into each photobioreactor. The PAR ranged from 11 to 450 µE m^{-2} s^{-1}. The pH was controlled by injecting CO_2 to acidify the media as required and the temperature was controlled. The pH ranged from 6 to 10 and the temperature ranged from 15°C to 35°C. The temperature, irradiance and pH were set to encompass the natural and culture conditions encountered under the Mediterranean climate and to allow a better definition of the optimal culture conditions for the microbial consortium. Continuous air bubbling (0.1 L min^{-1}) was provided for culture homogenization and preventing sedimentation.

2.3. Chemical measurements

To determine the acetate concentrations, 2 ml subsamples were collected every 2 days and filtered through a GF/F filter (Whatman) to remove particulate matter and potential consumers and stored in the dark at 4°C. Acetate concentrations were then measured within 2 days using gas chromatography (GC-8000 Fisons instrument), equipped with a flame ionization detector (temperature of the oven and the detector were 120°C and 280°C, respectively). The maximum injection temperature was 250°C and the regeneration temperature was 200°C. An internal standard was used for quantification of the acetate concentrations.

Subsamples were collected at the beginning and end of the experiment in order to determine the total carbon and nitrogen content of microbial assemblage (i.e. total C and N biomass). 3 ml of the subsamples were filtered through precombusted (500°C for 5 h) Whatman GF/F glass-fiber filters. The filtered samples were dried at 60°C for 24 h, pelleted and analyzed for total carbon and nitrogen content using an Integra CN elemental analysis-mass spectrometry system (PDZ Europa Scientific, UK) and calibrated with glycin references every batch of 10-15 samples.

2.4. Biological measurements

2.4.1. Optical density

The optical density at 680 nm (OD) was measured as a proxy for biomass (Hulatt & Thomas, 2010). 300µl samples were collected from each photobioreactor and immediately analyzed using a spectrophotometer. A linear relationship between OD and Besides total C content only measured at the beginning and at the end of the experiment, total C content (expressed in mgC L^{-1}) was determined along the 10d experiment for this consortium (R^2 = 0.94, n = 18, p < 0.0001, Figure 1) giving equation (1) :

$$\text{Total C content} = 266.06 \times OD + 7.34 \quad (1)$$

2.4.2. Microalgae abundance and carbon content conversion

For microalgae abundance, 1.6 ml samples were collected daily from each culture and were fixed with formaldehyde (2% final concentration) and frozen at -80ºC prior to analysis using a FACSCalibur flow cytometer (Becton Dickinson) equipped with an air-cooled argon laser (488 nm, 15 mW). Samples were thawed and beads of 2, 6, 10 and 20 µm diameter (YG polysciences) were added as internal fluorescence and size standards. The flow rate was calibrated using Trucount beads (ca. 4.5 µm, Becton Dickinson). Microalgae cells were detected using the red chlorophyll pigment fluorescence (>650 nm). The data were analyzed using Cell Quest Pro software (Becton-Dickinson). The abundances were converted into carbon content using the conversion factor of 2 pgC $cell^{-1}$ for *Nannochloris* (DuRand & Olson, 1998).

2.4.3. Bacterial abundance and carbon content conversion

For bacterial abundance, 1.6 ml subsamples were collected daily from each culture, fixed with formaldehyde (2% final concentration) and frozen at -80 ºC prior to flow cytometric analysis. Samples were then thawed at room temperature and using an anionic detergent, Tween 80, was used to release bacteria attached to microalgal cells and aggregates. Tween 80 (0.2% final concentration) was added to 500 µl aliquots of the samples. The samples were mixed for 1 min and incubated for 15 min at room temperature. The bacteria were detached physically by 15 min sonication (75W) in an ice bath to avoid heating the samples. Three hundred microliters aliquots of sonicated culture were sub-sampled and stained as described below.

The nucleic acids in the bacterial samples were stained for 15 min in the dark at room temperature with SYBR Green I (1:10,000 vol:vol, Molecular Probes). The bacteria were enumerated using the FACSCalibur flow cytometer detecting the 530 nm green fluorescence (FL1) of the stained nucleic acids of each bacterial cell. 1-µm and 2-µm yellow–green fluorescent cytometry beads (Polysciences) were added to the samples as internal standards for cell size and fluorescence emission, and TruCount control beads (Becton Dickinson) were added to determine the volumes analyzed.

The bacterial carbon content was calculated using a conversion factor of 30 fgC $cell^{-1}$ for productive waters (Fukuda et al. 1998).

2.4.4. Estimation of carbon conversion efficiency and growth rates

The carbon conversion efficiency was calculated using equation (2):

$$CE (\%) = (C\ content\ Produced / C\ Supplied)*100 \quad (2)$$

Where the C content Produced was the difference between the maximal or final carbon content estimated for each biological compartment during the incubation period. C Supplied was the initial concentration of acetate in the culture medium and/or the maximum photosynthetically fixed CO_2. The maximum photosynthetic fixed CO_2 was calculated from the light energy received during the whole incubation period and using the calorific content of phytoplankton factor of 541 kJ $molC^{-1}$ (Williams and Laurens 2010).

The specific growth rate (μ, d^{-1}) was calculated according to the equation (3)

$$\mu = \ln (Ct - C0) / (t - t0) \quad (3)$$

where Ct and C0 are the abundances at the end and the beginning of the exponential phase, respectively, and t-t0 is the duration of the exponential phase (Jiang et al., 2011).

2.4.5. Bacterial diversity

In each PBR, 1.5 ml subsamples for bacterial diversity were collected daily. Each sample was immediately centrifuged for 5 min at 3000 g. The pellets were kept frozen at -80°C prior to analysis. The bacterial diversity was determined by Capillary Electrophoresis-Single Strand Conformation Polymorphism (CE-SSCP) analysis. The universal 16S rRNA gene primers W49 (ACGGTCCAGACTCCTACGGG, *Escherichia coli* position F331) and 5'-fluorescein phosphoramidite labeled W104 (TTACCGCGGCTGCTGGCAC, *E. coli* position R533) were used (Delbes et al., 2001). Each PCR mixture (50 µl) contained 1X *Pfu* Turbo DNA polymerase buffer, 200 µM of each dNTP, 130 ng of each primer, 0.5 U of *Pfu* Turbo DNA polymerase (Stratagene) and 1 ng of genomic DNA. PCR was carried out in a Mastercycler thermal cycler (Eppendorf). The 16S rRNA genes were amplified at 94°C for 2 min, followed by 25 cycles performed at 94°C for 30 s, 61°C for 30 s and 72°C for 30 s, with a final extension at 72°C for 10 min. The size of the PCR products (200 bp) was assessed by 2% gel electrophoresis and staining with ethidium bromide. 1µl of diluted PCR products was mixed with 18.8 µl of formamide and 0.2 µl of internal standard GeneScan ROX (Applied Biosystems). Samples were heat-denatured at 95°C for 5 min and immediately cooled in ice. CE-SSCP electrophoresis was performed in an ABI Prism 3130 genetic analyzer (Applied Biosystems) with 50 cm long capillary tubes filled with a non-denaturing 5.6% conformation analysis polymer (Applied Biosystems). Samples were eluted at 12 kV and 32°C for 30 min. The CE-SSCP profiles were aligned with the internal standard to take account of inter-sample electrophoretic variability. The CE-SSCP profiles were normalized using the StatFingerprints R library (Michelland et al., 2009) using a standard procedure (Fromin et al., 2002). The Simpson diversity index was estimated using the default parameter of StatFingerprints, which takes into account the number of species (number of peaks) as well as their relative abundance (area under each peak).

Bacteria populations were identified using 16S rDNA phylogeny. Partial 16S rDNA sequences were amplified and cloned in TOPO® TA Kit (Invitrogen), and then twenty clones were selected for sequencing. Sequences were aligned and the phylogenetic trees were constructed using the neighbor-joining method. Distance matrices were calculated using Kimura's 2-parameter distances. The robustness of the topologies was assessed by the bootstrap method with 500 replicates.

2.5. Statistical test

A systematic experimental method to optimize total and microbial biomass was designed using Central Composite Design (CCD). Temperature (T), irradiance (I), and pH were studied as the three independent variables. Statgraphics (StatPoint Technologies, Inc. (USA) was used for regression analysis and analysis of variance (ANOVA). All statistics were considered significant at $p<0.05$.

3. Results and discussion

Very few studies have evaluated the biomass production and accumulation of marine algae-bacteria consortia within a biotechnological context (Fouilland 2012). Selected marine microalgae cultivated using wastewaters or fertilizers showed maximal growth rates varying between 0.15 to 0.54 d^{-1} and a maximal abundance reaching 1×10^6 and 55×10^6 Cell mL^{-1} for *Chaetoceros coarctatus* and *Nannochloropsis*, respectively (Jiang et al., 2011; Kim & Jeune 2009 ; Rocha et al., 2003; Venkatesan et al., 2006). The maximal growth rate (0.72 d^{-1}) and the maximal cell abundance (70.2×10^6 Cell mL^{-1}) observed in the present study (Table 1) were greater than previously observed with marine microalgae.

The influence of three variables (temperature, T; irradiance, I and pH) on the microbial community biomass and diversity was examined. The experiment lasted for 10 days allowing the microalgae mass to reach its maximum carrying capacity. The light, temperature and pH conditions used in this study, detailed in Table 1, were selected to cover a wide range of natural climatic and culture conditions (15°C < T < 35°C; 11 $\mu Em^{-2}s^{-1}$ < I < 450 $\mu Em^{-2}s^{-1}$; 6< pH < 10).

3.1. Dynamics of the microalgal C content

The maximum C content of *Nannochloris* spp varied between 16.7 to 165 mgC L^{-1} (Table 1) and was observed on average after 8 ± 1 days of incubation (Fig. 2a). It was always greater than the initial C content concentration (14.6 mgC L^{-1}). The highest value was measured under the highest light conditions (450 $\mu E\ m^{-2}\ s^{-1}$, PBR 4). Irradiance and temperature had a statistically significant effect on the maximum carbon content (Table 2). High pH conditions did not seem to have a strong influence on microalgal C content production. The optimal conditions predicted for the maximum C content (134.5 mgC L^{-1}) were close to those for PBR 4 which had the highest level of light supplied (Table 1).

The microalgal C conversion efficiency (equivalent to the photosynthetic efficiency, i.e. the conversion of light energy into microalgal carbon content) ranged between 0.03% and 3.64%, and with a mean of 0.52 ± 0.82% over the 17 conditions. The highest C conversion efficiency was measured at the lowest light intensity (11 $\mu E.m^{-2}.s^{-1}$) and the lowest C conversion efficiency was observed at the highest temperature (31°C) and pH (9.2), resulting in the lowest final algal C content observed. The optimized microalgal C conversion efficiency was 4.0% (Table 3). The mean microalgal C conversion efficiency measured in the experiment was one order of magnitude lower than the theoretical maximum photosynthetic efficiency but was close to the empirical values reviewed by Williams & Laurens (2010). The range of C conversion efficiency observed in this study is in agreement with the photosynthetic efficiency of 1% observed by Hullatt & Thomas (2011) for an outdoor photobioreactor in a mid-temperate zone. These results suggest that marine microalgae using anaerobic digester effluent as a medium will have a similar microalgal C conversion efficiency to those in a more conventional culture medium.

3.2. Dynamics of the bacterial carbon and diversity using acetate

The bacterial carbon content was 0.6 mgC L^{-1} at the beginning of the experiment. A first peak of bacterial carbon content occurred within the first two days of the experiment in all the PBRs (Fig. 2B), coinciding with acetate exhaustion. The dissolved organic carbon potentially exudated by microalgae was considered negligible at the beginning of the incubation period. Prokaryotic diversity (in this case heterotrophic bacterial diversity as no cyanobacteria were observed) measured during the first peak of bacterial C content was 1.6 ± 0.4. This suggested that a specific group of bacteria grew on a single source of carbon. The bacterial community was dominated by γ-Proteobacteria; mainly *Pseudomonas stuzeri* and α-Proteobacteria related to *Labrenia*, *Hoefflea* and *Sulfitobacter*. Bacterial diversity seemed, therefore, to be constrained by the single organic carbon source that was available (Fig. 3).

The maximum bacterial C content observed during the first days of the experiment varied between 5.9 and 19.9 mgC L^{-1}. The bacterial C conversion efficiency based on acetate (i.e. the conversion of acetate into bacterial C content) ranged between 1% and 20%, being maximum when the temperature was 19°C at pH 6.8 and minimum when the temperature was 25°C at pH 10. The pH and temperature had a significant effect on the maximum bacterial C content from acetate. However, the optimum temperature and pH conditions were close to those for the maximum C content of *Nannochloris* spp. This suggested that both bacterial and phytoplankton C production may be optimal under similar growth conditions. The addition of acetate increased the mean microbial C conversion efficiency by 40% (i.e. conversion of light energy and initial acetate into C content of both *Nannochloris* and bacteria) in comparison with the mean C conversion efficiency based on microalgae only (Table 1).

Anaerobic digester effluents (ADE) may contain high quantities of volatile fatty acids (VFA). Of these, acetate is one of the major products of anaerobic fermentation of organic matter. Acetate is also preferentially consumed by heterotrophic bacteria in both freshwater and marine systems. The bacterial C content decreased rapidly after peaking, at acetate exhaustion (within 2 days) in this study, confirming the importance of acetate for the growth of this bacterial community. Previous mixed cultures of bacteria and one microalgae species were shown to be capable of simultaneous removal of organic acids, pollutants and inorganic elements (Tang et al., 2010). The association between microalgae and bacteria in mass cultures is, therefore, clearly important.

3.3. Dynamics of the bacterial C content and diversity associated with microalgae

A second bacterial C content maximum in the PBRs occurred towards the end of the experiment (Fig. 2B). This increase in bacterial C content followed the growth of *Nannochloris* spp, when the readily available carbon source was exhausted. This suggested that this bacterial community was more dependent on microalgal exudation at the end of incubation. This second maximum of bacterial C content was observed on average 9 ± 1 days after the beginning of incubation. This maximum bacterial C content was assumed to depend directly on microalgae exudation and varied between 1.8 and 26.6 mgC L^{-1}. As for the maximum microalgal C content, irradiance and temperature were the two factors that had a significant effect on this second maximum bacterial C content. The optimum conditions for the maximum bacterial C content on exudates were obtained for the PBRs 2 and 4 corresponding to the highest microalgal

C content (Table 1). The optimized irradiance condition was similar to that obtained for the maximum C content of *Nannochloris* spp. However, the optimum conditions for the maximum bacteria C content on exudates suggested more light and higher temperature conditions than for the maximum bacterial C content on acetate (Table 2). These results suggest that this bacterial community was different from that observed during the first 2 days. The prokaryotic diversity increased from 1.6 ± 0.4 to 2.1 ± 0.4 from the first to the last incubation period. A drastic change in the prokaryotic community occurred during the experiment from the initially dominating *Pseudomonas* genus to the finally dominating genus Cytophaga, and Flavobacterium, closely related to the genus *Kordia*. The α-Proteobacteria group remained constant throughout the experiment (Fig. 3). During the culture period strong algal-bacterial coupling was observed as has been suggested for productive systems (Fouilland & Mostajir, 2010). Such coupling suggested that bacteria used organic carbon released by microalgae for their own growth. Microalgal exudates may represent a significant fraction of carbon fixed by the algae from 2% to 10% during exponential growth rising to 10% to 60% in the stationary phase. Hulatt & Thomas (2010) and reference therein reported that the release of algal dissolved organic matter (DOM) in natural systems could reach 80% of that fixed by photosynthesis. The presence of bacteria growing on microalgal exudates recycles various dissolved by-products, potentially lost by microalgae, into biomass and, therefore, reduces the loss of algal biomass productivity reported by Hulatt and Thomas (2010). Without introducing any other nutrient inputs, the presence of such bacteria increased the mean C conversion efficiency by 62% and 17% compared with the presence of (i) microalgae only and (ii) microalgae and bacteria using acetate, respectively (Table 1). The associated marine bacterial community could be, therefore, considered beneficial to the total C production, allowing a significant increase in C conversion efficiency.

3.4. Dynamics of the total C content and pH influence

Over the course of the 10 day experiment, the total C content increased from 29 mgC L^{-1} to a maximum of 380 mgC L^{-1} (Table 1). The total content C:N ratio was 6.8 ± 0.3 at the beginning of the experiment and ranged from 4.9 and 48.1 at the end of experiment at the lowest (6.8) and highest pH (10.0), respectively, (Table 1). The pH had a significant effect on the total C content and the C:N ratio (Table 2). When the pH was greater than 9 a well marked increase in total C content (>150 mgC L^{-1}) was measured (Table 1). The optimum conditions for the maximum total C content were close to those for PBR 15 and were characterized by very high pH (Table 2).

The high C:N ratio (up to 48.) measured in this study suggested that this total C content was probably largely exudated microalgal carbohydrates which precipitated and accumulated in particulate form at high pH. Chin et al (1998) showed that free marine DOM can aggregate to form particulate matter. They showed that an increase in seawater pH (from 8.5 to 9), as observed in microenvironments in conditions of high productivity, could explain the flocculation of marine DOM through the formation of organic marine snow and aggregates leading to an increase in sedimentation. The presence of a polysaccharide-enriched organic matter responsible for the adsorption of natural marine organic matter at pH 10 was also shown by Quigley et al. (2001). In this experiment, a pH above 9 induced a strong, rapid sedimentation of particulate matter (visual observation during the experiment). This suggested that artificially increasing the pH in marine cultures might greatly improve the recovery of particulate carbon and facilitate culture harvesting as has been suggested by other authors (Chen et al., 2011).

4. Conclusions

A marine algae-bacteria consortium dominated by *Nannochloris* spp growing on a synthetic anaerobic digester effluent reached a maximum C conversion efficiency of 3.6%. The efficiency reached 6.3% when the bacterial communities were included. This study showed the presence of various bacteria within the consortium improved the carbon conversion efficiency recycling the carbon lost during photosynthesis and originated from anaerobic digesters. Further investigations are required for assessing the effect of (i) various organic effluents on the C conversion efficiency of marine microbial consortia, and (ii) other components such as micronutrients, organic matter residues and turbidity which characterize the complexity of a real microalgae digestate.

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Table 1

Experimental conditions for the 3 variables: Temperature (T), irradiance (I) and pH. Experimental results for concentrations of C content, total content C:N ratio, microalgal abundance and growth rates, index of microbial diversity, and carbon conversion efficiency (%) obtained for i) microalgae, i) microalgae & bacteria on acetate, iii) microalgae & bacteria on acetate & bacteria on exudates, obtained after the 10 day experiment in the 17 photobioreactors. n.d. no data

PBR	**I**	**T**	**pH**	**Total C content (mg L^{-1})**	**Total content C:N ratio**	**Max microalgal C content (mgC L^{-1})**	**Max Bacterial C content on acetate (mgC L^{-1})**	**Max Bacterial C content on exudates (mgC L^{-1})**	**Max microalgal abundance (10^6 cell mL^{-1})**	**Microalgal growth rate (d^{-1})**	**Ln Simpson Prokaryotic**	**Ln Simpson Eukaryotic**	**Microalgal C conversion efficiency (%)**	**Microalgal + bacterial on acetate C converson efficiency (%)**	**Microalgal + bacterial on acetate and exudate C conversion efficiency (%)**
1	230	25	8	135.2	5.4	97.8	17.9	9.3	31.5	0.38	1.9	1.4	0.58	0.70	0.77
2	360	31	6.8	157.9	6.6	93,.4	18.1	30.7	0.5	0.55	1.9	2.4	0.40	0.49	0.65
3	230	25	6	82.3	5.9	65.0	9.6	4.9	21.4	0.47	2	1.4	0.31	0.37	0.40
4	450	25	8	173.3	6.9	165.6	11.2	26.6	67.8	0.43	2	n.d.	0.48	0.51	0.60
5	11	25	8	27.6	6.0	33.2	14.8	5.5	8.8	0.16	2.1	1.2	3.64	5.36	6.27
6	230	35	8	15.8	10.1	42.5	11.9	11.3	0.0	0.72	n.d.	n.d.	0.79	1.07	1.38
7	360	19	9.2	125.4	9.6	49.5	7.4	2.4	24.7	0.61	1.9	1.8	0.18	0.21	0.23
8	360	31	9.2	126.3	12.0	26.3	15.0	4.8	12.0	0.13	2.8	1.9	0.04	0.09	0.11
9	100	19	6.8	61.8	5.0	45.7	19.9	2.6	22.7	0.43	1.6	1.2	0.40	0.64	0.68
10	230	25	8	87.6	5.2	76.1	19.6	3.5	29.1	0.39	2.1	1.3	0.38	0.50	0.52
11	100	31	9.2	149.0	24.1	16.7	14.3	6.6	4.3	0.18	3.2	0.9	0.03	0.25	0.36
12	100	31	6.8	48.1	6.3	35.0	13.2	7.3	70.2	0.19	2.2	n.d.	0.26	0.42	0.51
13	230	15	8	69.3	5.5	54.9	5.9	4.2	24.6	0.40	1.9	1.1	0.28	0.32	0.35
14	360	19	6.8	83.9	4.9	80.0	12.4	1.8	29.3	0.47	1.5	1.3	0.26	0.31	0.31
15	230	25	10	380.2	48.1	43.0	1.4	4.3	9.1	0.45	2.6	2.2	0.18	0.18	0.21
16	230	25	8	110.0	6.7	75.1	18.2	17.2	35.0	0.29	2.2	1.7	0.34	0.44	0.53
17	100	19	9.2	60.7	10.5	27.2	8.4	1.9	7.9	0.27	n.d.	n.d.	0.23	0.37	0.40

Table 2

Optimized T, I and pH conditions maximizing C content of each microbial compartment.

* Variable having a significant effect.

Optimization	**I**	**T**	**pH**	**optimum**	**R^2**
Microalgal C content (mgC L^{-1})	450.0 *	24.7 *	7.5	134.5	78.6
C content of bacteria on acetate (mgC L^{-1})	200.3	25.9 *	7.6 *	18.8	76.2
C content of bacteria on exudate (mgC L^{-1})	450.0 *	35.0 *	6.4	48.9	76.5
Total C content (mgC L^{-1})	450.0	22.1	10.0 *	331.5	69.8

Table 3

Optimized T, I and pH conditions maximizing the C conversion efficiency for each microbial compartment .

* Variable having a significant effect.

Optimization	**I**	**T**	**pH**	**optimum**
Microalgae C conversion efficiency	11.0 *	25.0	8.0	4.0
Microalgae + bacteria on acetate bacteria C conversion efficiency	11.0 *	25.0	8.0	4.9
Microalgae + bacteria on acetate and exudate C conversion efficiency	11.0 *	25.0	8.0	5.4

Figure captions

Figure 1: Relationship observed between Total Carbon content (mgC L^{-1}) and Optical Density measured at 680 nm (OD 680) during the experiment for the whole data set.

Figure 2: Temporal variations of the Carbon content (mgC L^{-1}) during the 10 day experiment for: **A** Microalgae, **B** Bacteria and **C** Total C content. Minimal (min), maximal (max) and mean values measured under the 17 experimental conditions.

Figure 3: Neighbor-joining phylogenetic tree of 16S-rDNA sequences from bacterial communities during the first (day 2) and last period (day 10). Sequences of the last period are shown in bold. Bootstrap values are expressed as a percentage of 100 replicates.

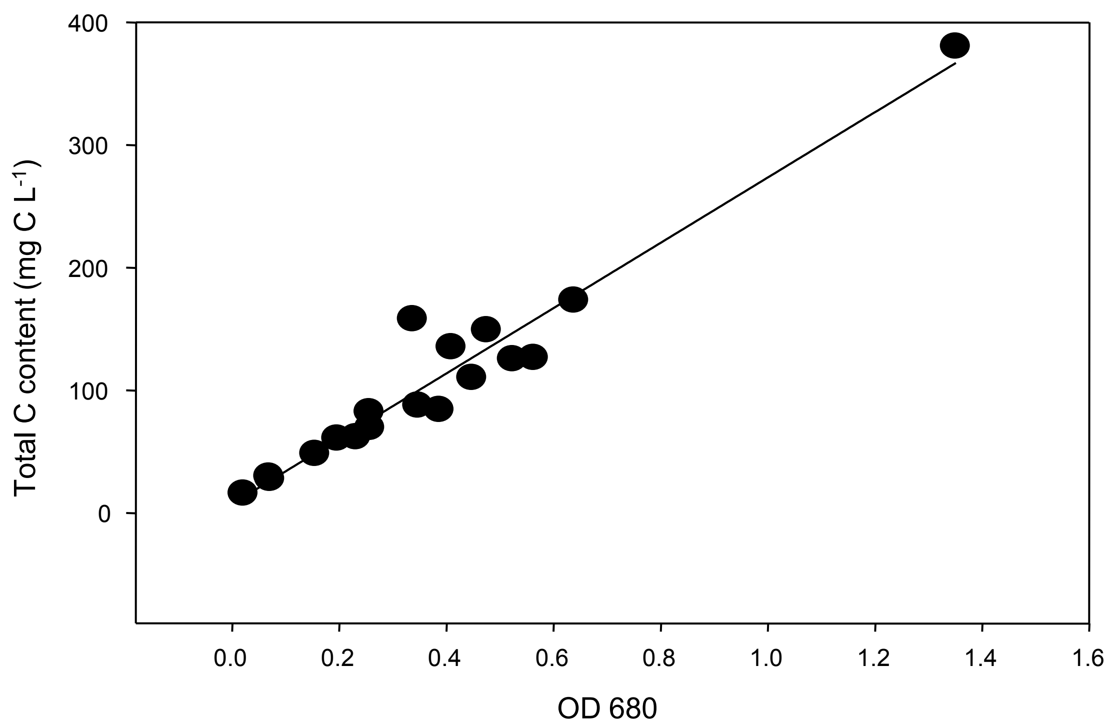

Figure 1

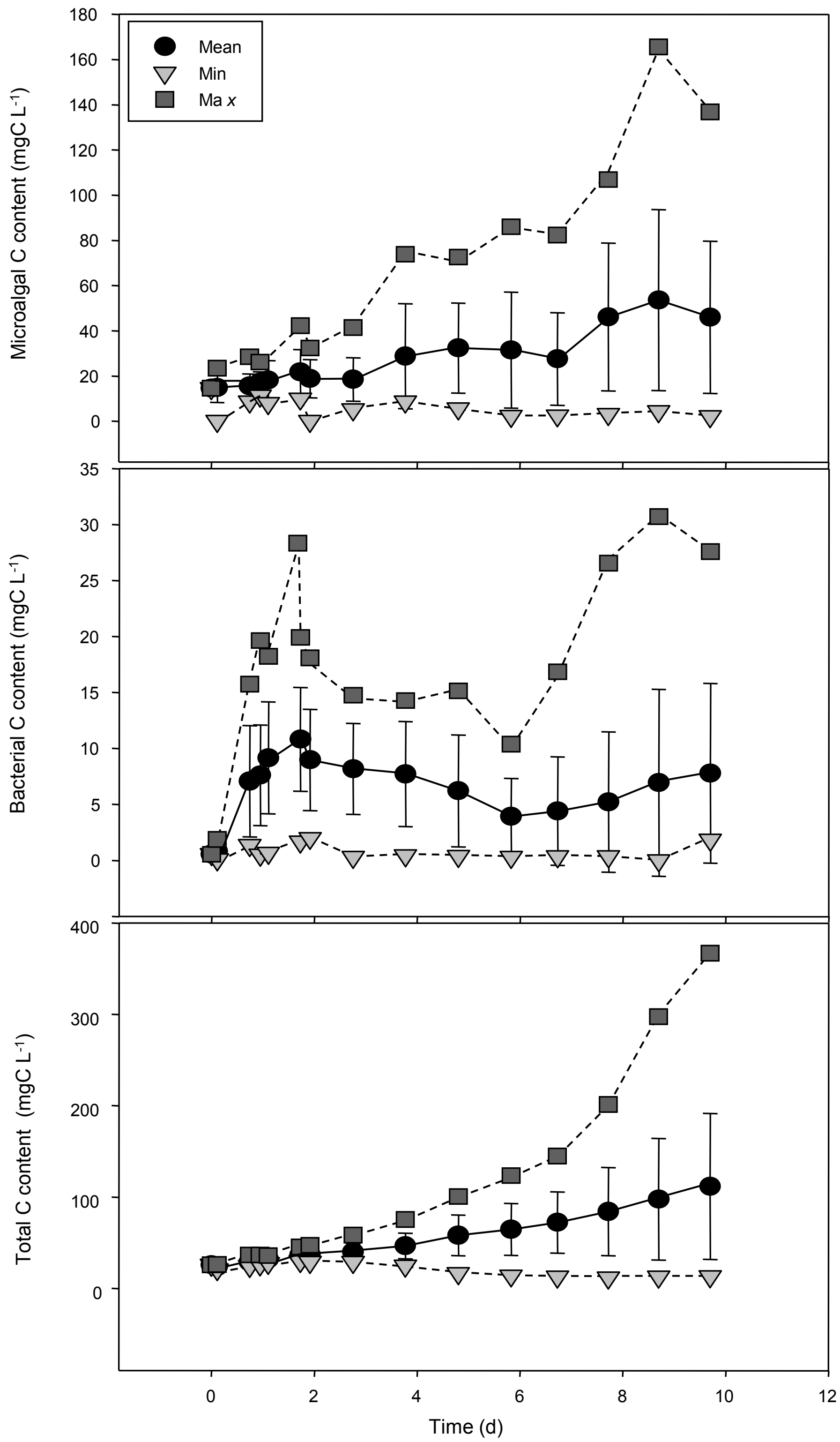

Figure 2

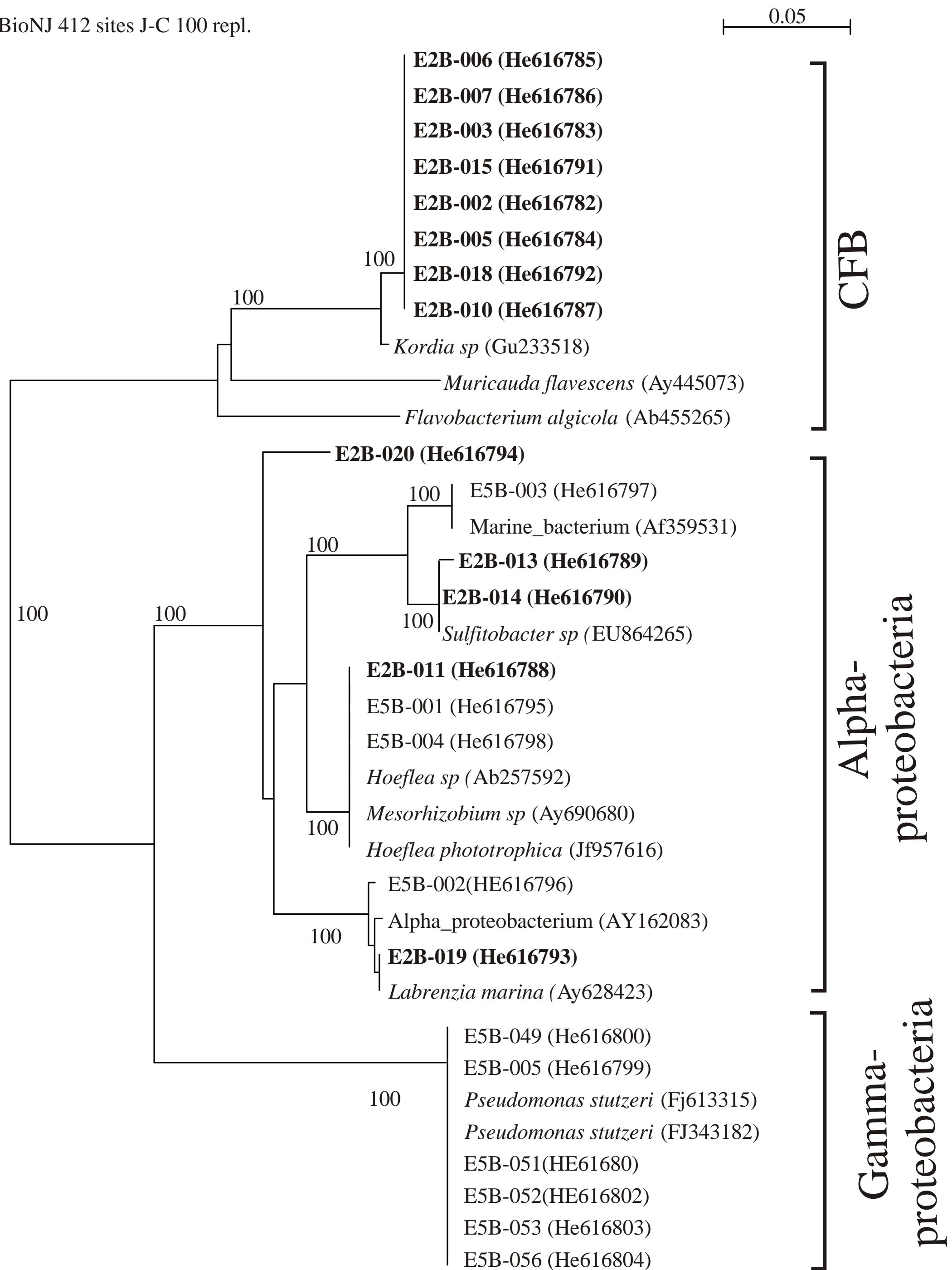

Figure 3