Bioresource Technology 363 (2022) 127979

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Centrate as a sustainable growth medium: Impact on microalgal inocula and bacterial communities in tubular photobioreactor cultivation systems

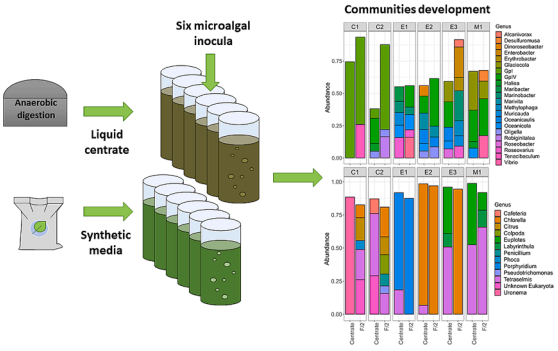
Elisa Clagnan [a], Giuliana D'Imporzano [a,*], Marta Dell'Orto [a], Alessia Bani [a,b,c], Alex J. Dumbrell [b], Katia Parati [c], Francisco Gabriel Acién-Fernández [d], Agustín Portillo-Hahnefeld [e], Antera Martel-Quintana [e], Juan Luis Gómez-Pinchetti [e], Fabrizio Adani [a]

[a] Gruppo Ricicla labs., Dipartimento di Scienze Agrarie e Ambientali – Produzione, Territorio, Agroenergia (DiSAA), Università degli studi di Milano, Via Celoria 2, 20133, Italy
[b] School of Life Science, University of Essex, Wivenhoe Park, Colchester, Essex CO3 4SQ, UK
[c] Istituto Sperimentale Lazzaro Spallanzani, loc La Quercia, 2602 Rivolta d'Adda, CR, Italy
[d] Department of Chemical Engineering, CIESOL Solar Energy Research Centre, University of Almeria, Cañada San Urbano, s/n, 04120 Almeria, Spain
[e] Spanish Bank of Algae (BEA), Institute of Oceanography and Global Change (IOCAG), University of Las Palmas de Gran Canaria, Muelle de Taliarte s/n, 35214 Telde, Canary Islands, Spain

HIGHLIGHTS

- Centrate is an efficient substitute for synthetic fertilizer.
- Centrate biomass showed higher N than synthetic media biomass.
- Dynamics and growths between media were comparable.
- Centrate might lead to higher richness, diversity and N metabolism genes abundances.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Microalgae
Centrate
Vertical bubble-column photobioreactor
Waste management
Next generation sequencing, Bacteria

ABSTRACT

Centrate is a low-cost alternative to synthetic fertilizers for microalgal cultivation, reducing environmental burdens and remediation costs. Adapted microalgae need to be selected and characterised to maximise biomass production and depuration efficiency. Here, the performance and composition of six microalgal communities cultivated both on synthetic media and centrate within semi-open tubular photobioreactors were investigated through Illumina sequencing. Biomass grown on centrate, exposed to a high concentration of ammonium, showed a higher quantity of nitrogen (5.6% dry weight) than the biomass grown on the synthetic media nitrate (3.9% dry weight). Eukaryotic inocula were replaced by other microalgae while cyanobacterial inocula were maintained. Communities were generally similar for the same inoculum between media, however, inoculation with cyanobacteria led to variability within the eukaryotic community. Where communities differed, centrate

* Corresponding author.
E-mail address: Giuliana.DImporzano@guest.unimi.it (G. D'Imporzano).

https://doi.org/10.1016/j.biortech.2022.127979
Received 3 August 2022; Received in revised form 11 September 2022; Accepted 12 September 2022
Available online 17 September 2022

resulted in a higher richness and diversity. The higher nitrogen of centrate possibly led to higher abundance of genes coding for N metabolism enzymes.

1. Introduction

Microalgae, especially Cyanobacteria, are labelled as "cell factories" thanks to their fast growth rates, high biodiversity and the enormous variety of bioactive substances and high-value products which they can produce. Microalgae are considered both a sustainable and economical source of renewable energy and a basis for agrochemical, biopharmaceutical and nutraceutical applications (Merlo et al., 2021). Recent works (Morillas-España et al, 2022; González-Pérez et al., 2022; Romero-Villegas et al., 2021) have highlighted an interest in the development of biostimulant products derived from microalgae, an application with high added value and which does not present the problems related to the food supply chain, especially when working on the recovery of nutrients from waste streams (e.g. slurry, wastewater, centrate and byproducts).

Commercial demand has pushed microalgal production towards the development of farming facilities using synthetic growth media to increase yields (Kawai et al., 2016). Cultivation with synthetic media, however, comes with high costs, and the use of organic fertilizers and wastewater (WW) has emerged as a low-cost alternative that also reduces the environmental burdens of WW discharge and gaseous emissions (Pahunang et al., 2021). WW comes from multiple sources (i.e. agricultural, municipal and industrial) and, due to its complex and variable composition, it requires characterisation to investigate how each compound can affect microalgal cultivation (Ge et al., 2018). On the other hand, centrate, which is the liquid phase resulting from the dewatering of anaerobic treated sludge, maintains the high nutrient content, therefore making it an efficient substitute for both WW (due to centrate higher P and N content) and synthetic media (because of the lower production costs of centrate). In the context of sustainability and circular economy, the use of centrate can be further advantageous to reduce the costs from its downstream remediation within the treatment plant (Sepúlveda et al., 2015; Ledda et al., 2015).

The use of centrate is however still limited mainly due to low microalgal tolerance at high NH_4^+ concentrations (Qiu et al., 2021). Romero-Villegas et al. (2018) showed that the community of a microalgal culture, its relative proportions, and nutrient removal efficiencies can be modified by changing the centrate concentration: however, different microalgal strains' tolerances and communities need to be investigated to find optimal conditions (Sepúlveda et al., 2015). Algal growth (e.g. *Chlorella, Scenedesmus* and *Nannochloropsis gaditana*) and bioremediation efficiency have been tested in previous studies on centrate (Lu et al., 2018; Ledda et al., 2015). However, native strains of microalgae that are adapted and can thrive when grown on different sources and concentrations of centrate need to be selected and studied to maximise production and depuration (Ge et al., 2018). Microalgae-bacteria consortia and bacterial participation also need characterisation, as they have been shown to improve WW treatment when compared to pure strains cultivation, since the community can perform multiple functions which can be difficult or impossible for a singular microorganism (Ayre et al., 2021).

The aim of this study was therefore to further understand how the use of centrate, as a sustainable alternative to synthetic growth media, can impact, maintain and sustain the growth of various microalgal inocula and their bacterial community structures in order to carry out the necessary monitoring of the population of new native consortia and comparing their populations. To achieve that aim, (1) six different microalgae inocula were cultivated in two different media (half-diluted F medium (F/2) and seawater diluted centrate) within tubular photobioreactors (PBRs), (2) their performance in biomass production was compared between media and (3) both bacterial and eukaryotic composition of microbial communities were investigated through 16S and 18S rRNA genes Next Generation Sequencing (NGS). Additionally, (4) the ability of the different inocula to remain pure, thanks to the high adaptation to the local environment, was further tested, as the five strains of this study, two cyanobacteria and three green microalgae, were selected based on their adaptation to the outdoor environment and growth conditions.

The results of this study will show the effect of the switch from synthetic fertilizer to centrate as the nutrient source on multiple communities and their stability within the perspective of bioremediation and high value products production.

2. Materials and methods

2.1. Experimental microalgae production

Experimental microalgae cultures were grown outdoors at the facilities of the Spanish Bank of Algae in Taliarte (27°59′27.7″ N, 15°22′07.4″ W; Gran Canaria, Canary Islands, Spain). A series of vertical bubble-column PBRs (volumes: 100 L) operated in continuous mode at 0.2 d^{-1} dilution rate, were used for scaling up and biomass production at the same site. The vertical bubble-column design was selected as it was readily available at the experimental site and as it is commonly used in both research and industrial set-ups from desk to full-scale plants with high production performances. Constant forced aeration was provided with a CO_2 pulse of 1.5 % CO_2-enriched air for 5 min per hour during the diurnal light-time. PBRs' temperature (determined by the environmental conditions), pH, salinity, and conductivity (determined by both CO2 delivery and microalgal growth) were measured with the portable Crison Instruments sensors, pH25 and CM25, previously calibrated. Irradiance was measured with a LI-4000 radiometer equipped with a spherical sensor SPQA 2770 (LI-COR, USA).

Cultures were grown under the same non-limiting conditions using two different growth media: half-diluted F medium (F/2; Total Kjeldahl Nitrogen (TKN): 88 ± 5 mg L^{-1}; N in the form of nitrate) (Guillard and Ryther, 1962) and 5 % seawater-diluted centrate, to achieve a final TKN of 270 ± 50 mg L^{-1} (mainly in the form of ammonium). The 5 % centrate concentration was selected after preliminary tests, as it was the centrate concentration assuring non-limiting nutrient conditions. The synthetic medium F/2 was selected as it ensures microalgal growth with its optimal nutrient concentration and was previously used in multiple experiments in comparisons to other media such as WW (Hawrot-Paw et al., 2020). Centrate was collected from the wastewater treatment plant at nearby Las Palmas de Gran Canaria, after the centrifuge step that separates the solid from the liquid fraction of the activated sludge.

PBRs were inoculated at 50 mg DW (dry weight) L^{-1} with six previously scaled up cultures of strains selected from the Culture Collection at the Spanish Bank of Algae (BEA) according to their growth characteristics and their robustness when inoculated under outdoor environmental conditions. Selected strains were two cyanobacteria: C1 - *Anabaena* sp. (BEA0912B) and C2 - *Dolichospermum* sp. (BEA0866B); three eukaryotic microalgae cultures: E1 - *Chrysoreinhardia giraudii* (Ochrophyta, BEA0313B), E2 - *Parachlorella* sp. (Chlorophyta, BEA0046B) and E3 - *Halochlorella rubescens* (Chlorophyta, BEA0069B); and a (1:1) mixed eukaryotic microalgal culture: M1 - *Chrysoreinhardia giraudii* and *Halochlorella rubescens*.

Biomass samples from each PBR were harvested by filtration and/or centrifugation, washed with sterile distilled water, frozen and freeze-dried (Freezone 6L, Labconco, USA). Productivity was calculated as the rate of the daily biomass increase in g DW L^{-1} d^{-1} (Hempel et al., 2012).

2.2. Chemical analyses

Biomass samples of about 250 mg were used to detect the N concentration (% m/m), using an elementary analyzer (Elementar Rapid max N exceed) based on the analytical method of combustion by Dumas and equipped with a thermal conductivity detector (TCD).

Macro and microelement concentrations including Na, Mg, K, Ca, P, Mn, Fe, Cu, Zn, Cr, Co, Ni, As, Se, Mo, Cd, Pb were determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, Aurora M90 BRUKER), preceded by microwave assisted (Multiwave ECO, Anton Paar GmbH) nitric acid digestion of fresh samples.

2.3. 16S and 18S rRNA gene NGS and bioinformatics

Twelve samples (~20 mg) of freeze-dried biomass of the assayed microalgae strains, harvested 4 days after reaching the steady state, were processed for DNA extraction using the Biosprint 96 One-For-All Vet Kit (Qiagen) in association with the semiautomatic extractor BioSprint 96 (Qiagen) and MagAttract technology, according to the manufacturer's instructions in three technical replicates taken from one PBR per treatment. The yield of the purified DNA was quantified using Qubit (Invitrogen, Italy) while DNA purity was measured through Nanodrop (Invitrogen, Italy). Possible fragmentation was determined through gel electrophoresis 1 % (w/v) 1 × TAE agarose gels. DNA was then stored at −80 °C until further analyses. Prior to NGS, the DNA extracted from the triplicate were pulled together to reduce biases due to the extraction step.

Library for 16S and 18S marker gene were prepared following Illumina Protocol. For the 16S, the hypervariable V3-V4 region was amplified using the 341F and 805R primers (Herlemann et al., 2011) while for 18S, the V9 region was amplified using the 1389F and 1510R primers (Piredda et al., 2017) both modified with the required Illumina sequencing adaptors. 16S and 18S PCR amplification was performed on a total volume of 25 µl: 12.5 µl of appTaq RedMix (Appleton Wood ltd, UK), 1 µl of forward and 1 µl of reversed primers modified with Illumina over-hanger (10 uM) (IDT, Belgium), 2.5 µl of extracted DNA and 8 µl of PCR grade water (Merck, Germany). Thermal protocol for 16S gene was 95 °C for 3 mins followed by 30 cycles at 95 °C for 15 s, 57 °C for 15 s and 72 °C for 30 s, with a final extension step at 72 °C for 7 mins. For 18S marker gene the thermal protocol was 98 °C for 3 mins, followed by 30 cycles at 98 °C for 10 s, 56 °C for 30 s and 72 °C for 15 s with a final extension step at 72 °C for 7 mins. PCR products were cleaned using Agencourt AMPure XP PCR Purification beads (Beckman Coulter), following the manufacturer's instructions. 2.5 µl of purified PCR product was used in a short secondary PCR, to attach Nextera XT indices, in the presence of 2.5 µl of Nextera i5 and i7 index, 12.5 µl Appletonwood Taq and 5 µl of PCR water. Thermal cycling conditions consisted of an initial denaturation step of 3 min at 95 °C followed by 8 cycles each of 30 s at 95 °C, 30 s at 55 °C and 30 s at 72 °C followed by a final extension step of 5 min at 72 °C. PCR products were purified using Agencourt AMPure XP PCR Purification beads as described previously. PCR products were quantified using PicoGreen® dsDNA quantification assays (Thermo Fisher Scientific), on a POLAR star Omega (BMG Labtech) plate reader. Nextera XT amplicons were then pooled in equimolar concentration. The length of amplicons was verified with Agilent bioanalyzer DNA kit (Agilent, USA). Final quantification of the pooled amplicon library was determined with the NEBNext® Library Quant Kit for Illumina® (New England BioLabs) prior to sequencing on the Illumina MiSeq (2 × 300 bp) at University of Essex (UK). The nucleotide sequences generated and analysed are available at the NCBI SRA repository (BioProject accession number: PRJNA742566).

Amplicons were processed following the same protocol as in Dumbrell et al., 2016 for 16S while for the 18S a slightly modified protocol has been used (Bani et al., 2021). The resultant OTU sequences were assigned taxonomy using the Naïve Bayesian Classifier against the RDP database. 18S sequences followed the same bioinformatic workflow as above with the only difference that classification has been done using Blastn (version 2.8.1) algorithm against NCBI nucleotide database and taxonomy retrieved using Taxdump Repository.

Table 1
Operating parameters in the assayed PBRs at the steady-state growing phase during the experimental period.

Parameter	F/2	Centrate
pH	7.9–8.2	8.3–8.6
Conductivity (mS/cm)	52–53.3	51.5–52.6
Temperature (°C)	21.1–25.7	21–25.9
Salinity (psu)	37.1–37.7	37–37.3
Irradiance (µmols photons $m^{-2}s^{-1}$)	512–1983	512 – 1983

2.4. Statistical analyses

All statistical analyses were performed on R studio (version 4.1.2) mainly with the package vegan (Oksanen et al., 2020) while taxonomic summaries were performed using the phyloseq package (McMurdie and Holmes, 2013). Observed richness, Simpson and Shannon diversity index were calculated and following a Shapiro-Wilk test to test normality, differences among samples of normally distributed data were tested by one-way analysis of variance (ANOVA), followed by a Tukey's post hoc test, while non normal data were analysed through a non-parametric Kruskal-Wallis test followed by Dunn's Test for multiple comparisons. For pairwise comparison, T-test and Wilcoxon signed-rank test was used for normal and non normal data respectively. Multivariate analyses were performed on Operational Taxonomic Unit (OTUs) relative abundances. To test the effect of time and media on beta diversity, first, a nonmetric multidimensional scaling (NMDS) based on Bray-Curtis distances was applied and then results were confirmed through a PERMANOVA test. Furthermore, pairwise comparisons were carried out with the package 'pairwiseAdonis' (Martinez, 2020). The betadisper function was further used to understand variance followed by the simper function to understand the main differences in composition. Dendrograms were constructed through Simprof (actual Bray-Curtis) and co-occurrences with package cooccur (Griffith et al., 2016) to reveal intra- and inter-kingdom interaction.

3. Results and discussion

3.1. Reactor performance in PBRs

The selected strains for this study all have the added value of being economically important in terms of bioremediation, biostimulants, biomass and agricultural biofertilizer production. Cyanobacteria are known as important producers of antifungal and antibacterial compounds with *Anabaena* sp. showing a broad activity spectrum against plant fungal pathogens (Pawar and Puranik, 2008) and *Dolichospermum* sp. also showing ability as nanoparticle producer (Hamida et al., 2020) together with *Parachlorella* sp. (Kadukova et al., 2016). On the other hand, *Parachlorella* sp., *Chrysoreinhardia giraudii.* and *Halochlorella rubescens* are also marine microalgae that have the potential to be used as biostimulants, showing hormone-like activities (Khan et al., 2009).

The six microalgal inocula were grown both on a synthetic medium (F/2) and seawater-diluted centrate within different PBRs with the same set up under non-limiting conditions in terms of nutrients and conditions (Table 1). The same culture of starting inoculum for each microalga strain was used for both media to avoid variation depending on the initial community compositions (Bani et al., 2021).

Productivity was similar between centrate and F/2 medium for all the strains, *Chrysoreinhardia giraudii* showed values averaging at 0.075 g DW $L^{-1}d^{-1}$, while the highest productivity values were obtained for *Halochlorella rubescens* (av. 0.131 g DW $L^{-1}d^{-1}$) and *Parachlorella* sp. (0.083 g DW $L^{-1}d^{-1}$). Lower productivity values were shown by

Table 2
Nutrients concentration among biomasses.

	F/2		Centrate		Increase	
	N [%]	P [mg/g]	N [%]	P [mg/g]	N [%]	P [mg/g]
C1	6.03	4,186 (±1)	8.85	8,086 (±129)	46.60	93
C2	3.13	1,483 (±197)	6.76	8,241 (±1,214)	116.08	456
E1	3.90	3,617 (±190)	4.64	2,891 (±77)	18.88	−20
E2	3.55	2,513 (±174)	3.74	2,231 (±222)	5.38	−11
E3	3.66	2,565 (±11)	4.76	5,943 (±138)	30.08	132
M1	3.09	4,376 (±452)	4.76	5,749 (±730)	54.28	31
Av. (St. Dev.)	3.89 (±1.00)	3,123 (±1,051)	5.58 (±1.72)	5,523 (±2,382)	45.22 (±35.62)	113 (±162)

cyanobacteria strains (av. 0.05 g DW $L^{-1}d^{-1}$).

Microalgae cultivated on F/2 medium showed an N content between 3.09 % and 6.03 % while on centrate, N content was between 3.74 % and 8.85 % indicating an increased N (between + 5.38 % to + 116.08 %) in the biomass grown in centrate (*t*-test p-value: $p < 0.05$; a statistically significant p-value ($p < 0.05$) allows us to reject the null hypothesis that the variances of groups compared are equal) (Table 2). In F/2, N is mainly in the form of NO_3^- while in centrate, it is of NH_4^+. Although microalgae can utilize different forms of N (i.e. NO_3^-, NO_2^-, NH_4^+ or organic N), NH_4^+ is preferred, as its uptake require less energy and microalgae can furthermore inhibit other N forms' uptake, favouring NH_4^+ (Kumar and Bera, 2020). The higher concentration of N, also in the NH_4^+ form, could have enhanced its uptake within the centrate cultures, leading to higher N content than in the F/2 grown biomasses.

In terms of phosphorus (P), microalgae showed higher concentration (*t*-test: $p < 0.05$) within centrate (5523 mg g^{-1} (±2382 mg g^{-1})) rather than in F/2 (3123 mg g^{-1} (±1051 mg g^{-1})) (except for E1 and E2) with an average increase of + 113 mg g^{-1} in biomass.

Other micro- and macro-elements were similar between the two media (except for barium which was generally higher in centrate biomass (*t*-test: $p < 0.05$)) (see e-supplementary materials).

3.2. Eukaryotic community composition

The 18S rRNA gene sequencing resulted in a total number of reads between 7,266 ± 774 and 64,246 ± 22,939 with a final number after the DADA2 pipeline between 3,452 ± 956 and 30,953 ± 11,087 (see e-supplementary materials).

In general, eukaryotic communities inoculated with a eukaryotic microalga showed stability between media in terms of β-diversity. A higher difference between media was seen with cyanobacterial inocula, since there was no eukaryotic starting inoculum, the eukaryotic population was shaped by the environmental population that colonized the vehicle, in this case centrate.

Communities further showed similar richness and α-diversity, however the same inoculum communities that differed in composition between media (cyanobacterial inocula) showed a trend of higher richness and α-diversity in centrate.

Most samples were dominated by the *Chlorophyta* phylum, green algae mainly living in marine habitats, with a distinct abundance of *Ciliophora*, unicellular predators or heterotrophs, especially in centrate C1 samples. E1 samples were further characterised by the presence of the *Rhodophyta* phylum (red algae) (see e-supplementary materials).

At a genus level, cultures inoculated with E1, E2 and M1 seemed to maintain the same population across both F/2 and centrate media, while E3 cultures seemed to differ, possibly being more affected by the semi-open set-up of the reactor (Fig. 1). In E1 cultures, the inoculum species was not retrieved in either of the two cultures but the red alga *Porphyridium purpureum* was found dominant in both F/2 (relative abundance: 88 %) and centrate (71–75 %) with the further presence of *Tetraselmis* (16–23 %) in centrate. *Porphyridium purpureum* is a red alga important in the production of exopolysaccharides (Medina-Cabrera et al., 2020). E2 cultures were composed mainly of *Chlorella* both in F/2 (96.6–97 %) and centrate (88–94 %) with a higher percentage of *Tetraselmis* (5–9 %) in the latter. The main genera in M1 cultures were *Tetraselmis* (F/2: 59–71 %, centrate: 50–54 %) and *Euplotes* (F/2: 6–22 %, centrate: 45–49 %), periphytic microorganisms often feeding on bacteria and microalgae (Liu et al., 2020), with a small amount of *Labyrinthula* (7–13 %) in F/2, pathogens of multiple seagrass species (Sullivan et al., 2013). E3 cultures in F/2 showed the presence of *Chlorella* (94–96 %) as in E2 cultures, while cultures in centrate showed a similar pattern to M1 with a prevalence of *Tetraselmis* (50–52 %), *Euplotes* (32–38 %), and *Labyrinthula* (8–12 %). Both *Parachlorella* (E2) and *Halochlorella* (E3) genera were not found however; as in E1 cultures, genera from the *Chlorophyta* phyla were detected. Identification of *Chlorella* and genera within the *Chlorophyta* phylum can be however challenging due to the high variation of phenotypes and poor ability of the 18S rRNA gene to distinguish between closely related taxa (Krivinaa and Temraleevaa, 2020).

Cultures inoculated with Cyanobacteria showed a higher variability between media than those inoculated with eukaryotic macroalgae (Fig. 1). C1 cultures in F/2 showed a prevalence of *Tetraselmis* (14–36 %) and *Chlorella* (6–18 %). On the other hand, C1 grown on centrate showed an almost complete dominance by *Uronema marinum* (87–89 %), a protozoan ciliate known as a fish parasite. C2 in F/2 showed, similarly to C1, the dominance of *Chlorella* (12–63 %) and *Tetraselmis* (50–51 %) with the additional presence of *Pseudotrichomonas* (2–19 %), a free-living parabasalid, and *Penicillium* (0–11 %), an ascomycete genus of major importance for decomposition of organic materials and production of penicillin. Fungi are thought either to enhance microalgal growth by forming a symbiotic association, or to reduce growth by competing for nutrient availability (Watanabe et al., 2005). The presence of *Colpoda* (17 %), a bacterivore, was further detected in one of the F/2 C2 cultures while centrate C2 cultures were characterised by *Tetraselmis* (32–59 %) and *Cafeteria* (6–15 %), a globally distributed marine bacterivorous protist (Massana et al., 2021). Cyanobacterial cultures grown on centrate showed a higher abundance of organisms which possibly hinder algal growth.

Eukaryotic communities generally showed no differences in terms of richness (Observed: number of different species in an ecological sample and Chao1: richness estimate with more weight given to rare species) and alpha diversity (Shannon: accounting for richness and evenness and Simpson: importance given to evenness) between centrate and F/2 cultures (Table 3). However, E2 cultures showed lower eukaryotic diversity than M1 and the two cyanobacterial cultures: C1 and C2 (Shannon and Simpson: $p < 0.05$). Considering both inoculum and medium, C2 and E3 showed a higher richness and diversity on centrate medium rather than F/2 (Richness: $p < 0.0002$; Shannon: $p < 0.0005$) therefore when the communities showed a difference in composition between media, centrate seemed to lead to a higher richness and α-diversity.

Although *Chlorophyta* dominated mostly in all the reactors, species different from the inoculated ones could be found, providing the evidence that semi-closed systems do not ensure the maintenance of the original culture independently from the media used. However, for some species the use of centrate led to a higher number of species and

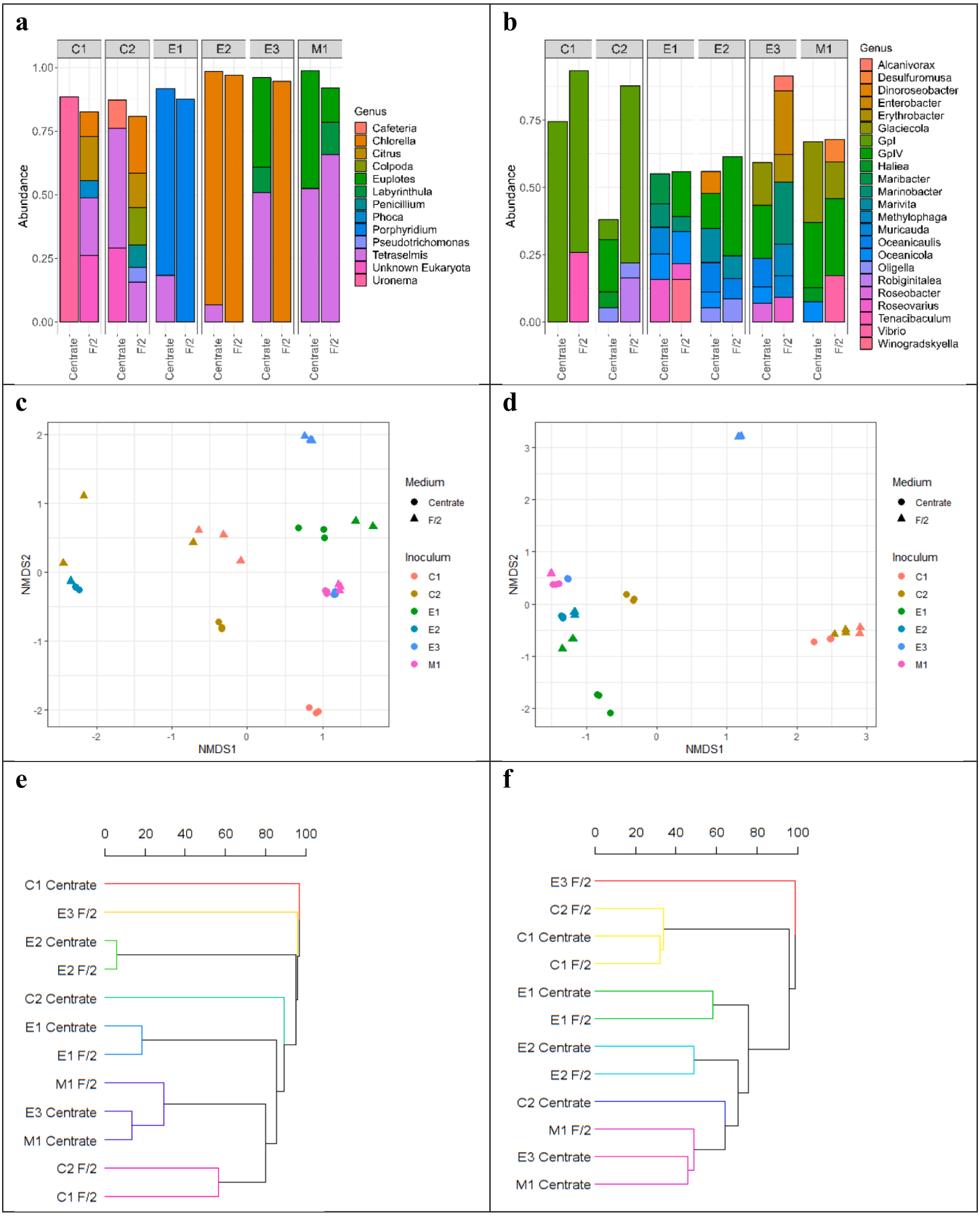


Fig. 1. Taxonomic composition at genus level of eukaryotic (a) and of bacterial (b) abundances (cut-off > 5 %) in each photobioreactor configuration. Average values of three replicates are shown for each bar. NMDS plot for the eukaryotic (c) and bacterial (d) community. Simprof cluster dendrogram based on Bray-Curtis dissimilarities for the eukaryotic (e) and bacterial (f) community, different colours indicate different clusters ($p < 0.05$).

Table 3
Eukaryotic and bacterial alpha diversity community indexes. Richness, Chao1, Shannon and Simpson: each number is the average of the three replicates (Av. (±St. Dev.)).

	18S				16S			
	Observed	**Chao1**	**Shannon**	**Simpson**	**Observed**	**Chao1**	**Shannon**	**Simpson**
Centrate								
C1	14 (±5)	16 (±6)	0.52 (±0.04)	0.21 (±0.01)	69 (±3.30)	84 (±3)	1.53 (±0.42)	0.48 (±0.13)
C2	36 (±3)	46 (±6)	1.92 (±0.13)	0.76 (±0.04)	166 (±53)	204 (±541)	3.99 (±0.04)	0.95 (±0.00)
E1	24 (±5)	27 (±7)	0.92 (±0.04)	0.43 (±0.01)	145 (±64)	174 (±57)	3.59 (±0.25)	0.95 (±0.01)
E2	12 (±2)	19 (±5)	0.34 (±0.10)	0.15 (±0.05)	99 (±7)	128 (±25)	3.37 (±0.05)	0.94 (±0.00)
E3	20 (±2)	24 (±2)	1.19 (±0.03)	0.62 (±0.01)	159 (±12)	218 (±8)	3.24 (±0.06)	0.91 (±0.00)
M1	24 (±7)	27 (±10)	0.87 (±0.03)	0.53 (±0.00)	178 (±47)	231 (±39)	2.99 (±0.06)	0.88 (±0.01)
F/2								
C1	21 (±5)	22 (±5)	2.13 (±0.15)	0.80 (±0.03)	13 (±2)	16 (±2)	0.85 (±0.12)	0.44 (±0.09)
C2	9 (±7)	10 (±7)	1.55 (±0.78)	0.67 (±0.17)	9 (±3)	9 (±3)	0.97 (±0.22)	0.43 (±0.10)
E1	22 (±1)	24 (±1)	0.61 (±0.01)	0.23 (±0.00)	78 (±44)	108 (±44)	3.12 (±0.49)	0.91 (±0.03)
E2	12 (±2)	14 (±3)	0.18 (±0.01)	0.06 (±0.00)	62 (±14)	78 (±12)	2.63 (±0.15)	0.83 (±0.02)
E3	12 (±1)	15 (±2)	0.44 (±0.03)	0.18 (±0.02)	40 (±12)	64 (±21)	2.20 (±0.05)	0.85 (±0.01)
M1	25 (±4)	45 (±16)	1.15 (±0.07)	0.53 (±0.05)	121 (±35)	144 (±32)	2.94 (±0.09)	0.88 (±0.01)

diversity, possibly due to the higher complexity of the medium, which may have sustained the development of a more complex and less selected community. The origin of external microorganisms "contaminating" the culture cause the weakest microalgae strains to be replaced by stronger ones with higher ecological advantages (Bani et al., 2021). Furthermore, the presence of organisms feeding on algae and bacteria (i.e. *Colpoda*, *Labyrinthula* and *Euplotes*) can be a further symptom of an unstable system that could potentially be subjected to failure risks (Molina-Grima et al., 2022).

3.3. Bacterial community composition

The 16S sequencing produced between 49,130 ± 18,926 and 13,548 ± 1,236 reads while after assembling, between of 38,163 ± 10,405 and 6,645 ± 600 (see e-supplementary materials).

Unlike the eukaryotic inocula, cyanobacterial inocula were always retrieved in the analysed samples. In terms of β-diversity, prokaryotic communities inoculated with cyanobacteria generally showed a higher similarity among them than the one presented for eukaryotic communities: this is also true for prokaryotic communities inoculated with eukaryotic microalgae. Community stability was again found between media, however centrate cultures again showed a higher richness and α-diversity when the communities showed a higher difference between the two media.

The bacterial taxonomic composition was dominated by three dominant phyla: *Proteobacteria* (5–93 %), *Cyanobacteria* (2–82 %) and *Bacteroidetes* (3–39 %) (see e-supplementary materials). *Proteobacteria* and *Bacteroidetes* phyla are characterised by generalist bacteria able to colonise various environments and often present in WW bioremediation and microalgae cultivation systems.

At a genus level, cultures inoculated with C1 maintained the same population across both F/2 and centrate media, while C2 in F/2 showed similarity to C1, but centrate C2 culture differed. E1, E2 and M1 again showed a similar bacterial community between F/2 and centrate, while E3 cultures seemed to differ, possibly due to external microorganisms entering the system due to the semi-open set-up (Fig. 1).

In C1 cultures cultivated in F/2 and centrate, the most abundant genus found was, as expected, *GpI* (*Anabaena* and possibly *Dolichospermum*) in both media (78–54 % and 78–56 % respectively), followed by *Tenacibaculum* (16–39 %) in F/2 and by *Alteromonas* (3–6 %) and *Roseovarius* (3–7 %) in centrate (Fig. 1). *Tenacibaculum* includes many opportunistic fish pathogens (Fernández-Álvarez et al., 2018) while *Alteromonas* are heterotrophic marine bacteria with oxidative metabolism (López-Pérez et al., 2014) and *Roseovarius* is a genus of the *Rhodobacteraceae* family involved in sulphur transformation and nutrient cycling (Shin et al., 2018). Similarly to C1, in C2 cultures the inoculum (*GpI*) dominated in F/2 (63–82 %) followed by *Robiginitalea* (7–18 %), a marine bacterium belonging to *Flavobacteriaceae,* and the genus *Oligella* (3–6 %) containing several human pathogens. By contrast, the centrate C2 culture did not show a dominance of the inoculated cyanobacteria *GpI* (6–7 %), but it showed a higher number of bacterial genera with thresholds<5 % and it was dominated by *GpIV* cyanobacteria (*Gloeobacter* and *Halomicronema*) (16–20 %) followed by *Oligella* (4–5 %) and *Mesonia* (4–5 %) another of the *Flavobacteriaceae*. Marine members of the *Flavobacteriaceae* family play key roles as degraders and in nutrient cycles and are often found in association with microalgal populations (Lucena et al., 2020).

Both E1 and E2 cultures showed similarities between the growth media. When looking at the cultures inoculated with eukaryotic microalgae, E1 in F/2 showed the dominance of the cyanobacterial genus *GpIV* (14–18 %) and the marine bacterial genera *Winogradskyella* (15–27 %), *Oceanicola* (10–14 %) and *Marivita* (3–8 %), while in centrate the prevalent genera were *Roseovarius* (12–20 %), *Maribacter* (11–12 %), *Muricauda* (6–12 %) and *Marinobacter* (8–11 %). E2 cultures showed, similarly to E1 in F/2, an abundance of *GpIV* (34–41 % and 12–14 % in F/2 and centrate respectively), *Marivita* (7–10 % and 12–14 %) and *Oceanicaulis* (7–8 % and 9–12 %) and *Oligella* (8.5–9.0 % and 5–6 %) with the addition, in centrate, of *Dinoroseobacter* (7–9 %) (genera containing phototrophic bacteria and algal symbionts (Wagner-Döbler et al., 2010)) and *Oceanicola* (5.5–6.3 %). E3 cultures showed a higher variability between media when compared to E1 and E2. When grown on F/2, E3 showed a predominance of *Enterobacter* (18–30 %), known to live predominantly in animals' guts, *Marinobacter* (19–26 %), *Methylophaga* (11–12 %) (a halophilic methylotrophic bacteria), *Erythrobacter* (9–11 %) (a marine photoheterotroph), *Roseovarius* (8–11 %), *Muricauda* (7–8 %) and *Alcanivorax* (5–6 %) a hydrocarbon-degrading marine bacterium (Cappello et al., 2007) while when grown on centrate, cultures were characterised by the presence of *GpIV* (19–21 %), *Glaciecola* (15–17 %), *Oceanicaulis* (9–13 %), *Roseobacter* (6–8 %) and *Oceanicola* (5.8–6.3 %). M1 cultures grown in both media showed high similarity to E3 grown on centrate with the most abundant genera such as *GpIV* (25–31 % and 23–27 %, in F/2 and centrate respectively) and *Glaciecola* (13–16 % and 29.9–30.7 %) with the addition in F/2 of *Vibrio* (16–18 %), known for including pathogenic species, and *Desulfuromusa* (7–10 %) a mesophilic sulphur reducer (Liesack and Finster, 2005) while on centrate, *Oceanicola* (6–8 %) and *Roseovarius* (4–5 %) occurred.

Cyanobacteria showed a high abundance as the main phylum in the cultures C1 and C2 but also in the communities not directly inoculated with Cyanobacteria (i.e. E1, E2, M1) possibly from advantages on other bacteria due to their photoautotrophic nature and not only due to their

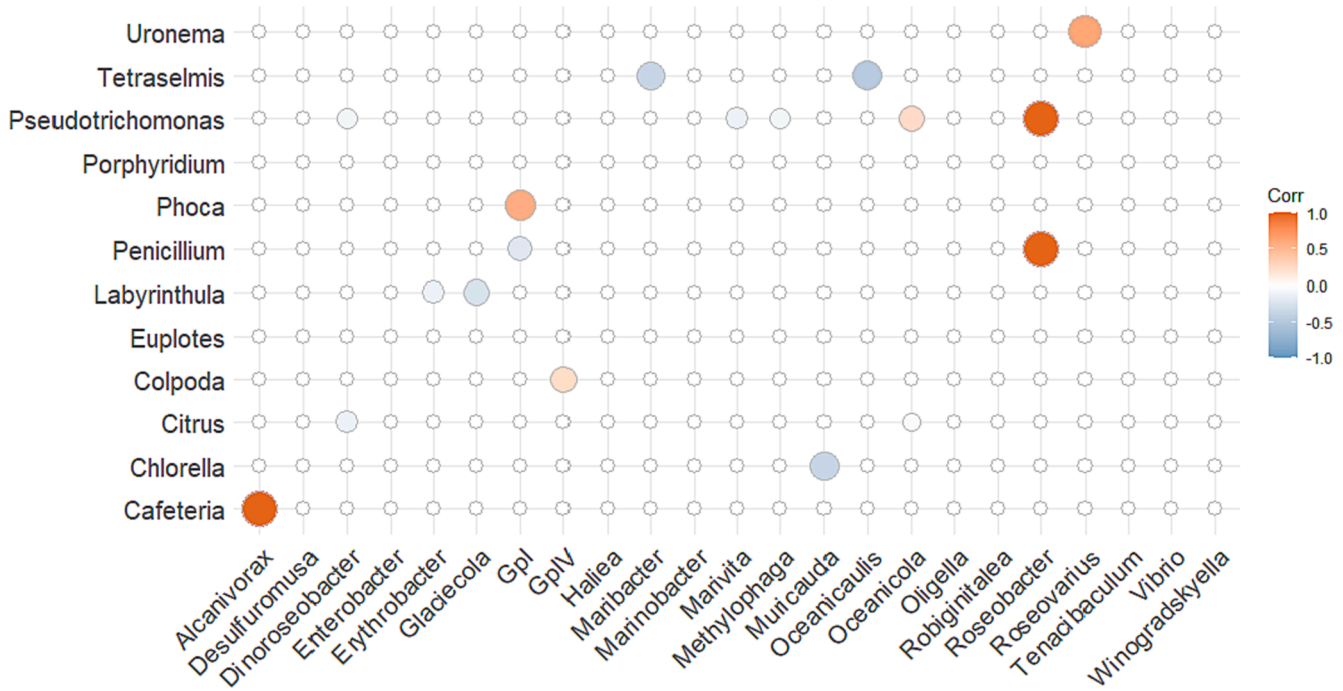


Fig. 2. Co-occurrence based on Spearman rank correlation index of the most abundant genera (>5%) for the statistically significant interactions (p value < 0.05).

specific inoculation (Singh et al., 2016). The presence of Cyanobacteria in most cultures highlights the ability of these photosynthetic prokaryotes to live and interact with eukaryotic microalgae in a synergic mode (Gautam et al., 2019).

Bacterial α-diversity indexes indicated a higher richness and diversity in centrate over F/2 (Richness: $p < 0.00005$, Chao1: $p < 0.00005$; Shannon: $p < 0.005$, Simpson: $p < 0.005$,) (Table 3). In terms of richness of the culture obtained from different inocula, the only difference was seen between C1 and M1 (Chao1: $p < 0.05$); however, C1 cultures showed a lower diversity than E1 cultures (Shannon and Simpson: $p < 0.005$) and than E2 (Shannon: $p < 0.05$). Considering both inocula and media, C2 and E3 showed higher richness (Richness: $p < 0.005$ and < 0.05, respectively; Chao1: $p < 0.0001$ and < 0.005) and diversity (C2 - Shannon and Simpson: $p < 0.05$) on centrate when compared to F/2. Prokaryotic communities were more diverse than the eukaryotic communities with a higher number of low abundance genera. Within the prokaryotic communities, the higher number of species and higher diversity were further enhanced within centrate cultures when compared to F/2.

3.4. Community interactions

β-diversity analyses indicated an influence of inocula, growth media and their interaction in shaping both prokaryotic and eukaryotic communities, with centrate leading to higher diversity and richness.

Both for eukaryotic and bacterial communities NMDS, cluster dendrogram and permanova analyses were used to test if two or more groups had similar compositions. They indicated an influence of inoculum, growth medium and their interaction on shaping the communities ($p = 0.001$) (Fig. 1). C1, C2 and E3 eukaryotic composition showed a higher difference between the two media than the other cultures in terms of community structure, while for bacterial composition, highest differences between the two media were exhibited again by C2 and E3. When analysing the samples that showed similar communities between media separately from the ones that showed different communities between media based on dendrogram analyses, permanova results were similar. When analysing in-depth the inoculum importance, permanova further showed significant differences between media within the same inoculum ($p = 0.001$) for both eukaryotes and bacteria. These results support the idea that each eukaryotic and bacterial inoculum can influence both bacterial and algal communities, as previously shown (Bani et al., 2021) and that centrate could lead to a higher diversity compared to the F/2 as a result of the higher species richness. However, within semi-closed reactors, contamination and cross-contamination are possible (see inoculum E3) and may affect the communities' dynamics: however, the use of a non-sterile medium such as centrate did not always lead to a significant change within the community.

Species composition was further investigated through a variances analysis to understand whether two or more groups had homogeneous dispersion. Eukaryotic communities showed significant differences in medium vs inoculum ($p < 0.001$) in both C1 and C2 cultures as variance differed between the two culture media ($p < 0.001$ and 0.05 respectively) (see e-supplementary materials). Therefore, differences between these two groups are most likely heavily influenced by their variance and differences (heterogeneous dispersion) in composition within them (and not by the difference in composition between groups). Main OTUs that contribute to these differences are *Uronema* and *Tetraselmis* for C1 cultures while *Tetraselmis*, *Chlorella*, *Pseudotrichomonas* and *Cafeteria* were important for C2 cultures. Inoculation without the eukaryotic microalgae (but with cyanobacteria) led therefore to higher variability in the development of the eukaryotic communities between media. On the other hand, bacterial communities only showed a significant difference when generally comparing medium ($p < 0.05$) and inoculum ($p < 0.001$), but did not vary significantly within each culture (see e-supplementary materials). Results were therefore most likely heavily influenced by a difference in composition among groups and not by their heterogeneity.

Finally, interactions between the eukaryotic and bacterial communities were investigated in terms of co-occurrence (Fig. 2). Among the most abundant genera, few interactions were detected, and the highest number of interactions was found for the eukaryotic *Pseudotrichomonas* (3 negatives and 2 positives). For bacteria, *Roseobacter* showed the highest number of positive interactions (2), while the photoheterotroph *Dinoroseobacter* of negative (2). The microalgae *Tetraselmis* showed only negative interaction with *Maribacter* and *Oceanicaulis*. *Chlorella* had a negative interaction with *Muricauda,* a genus known for quorum

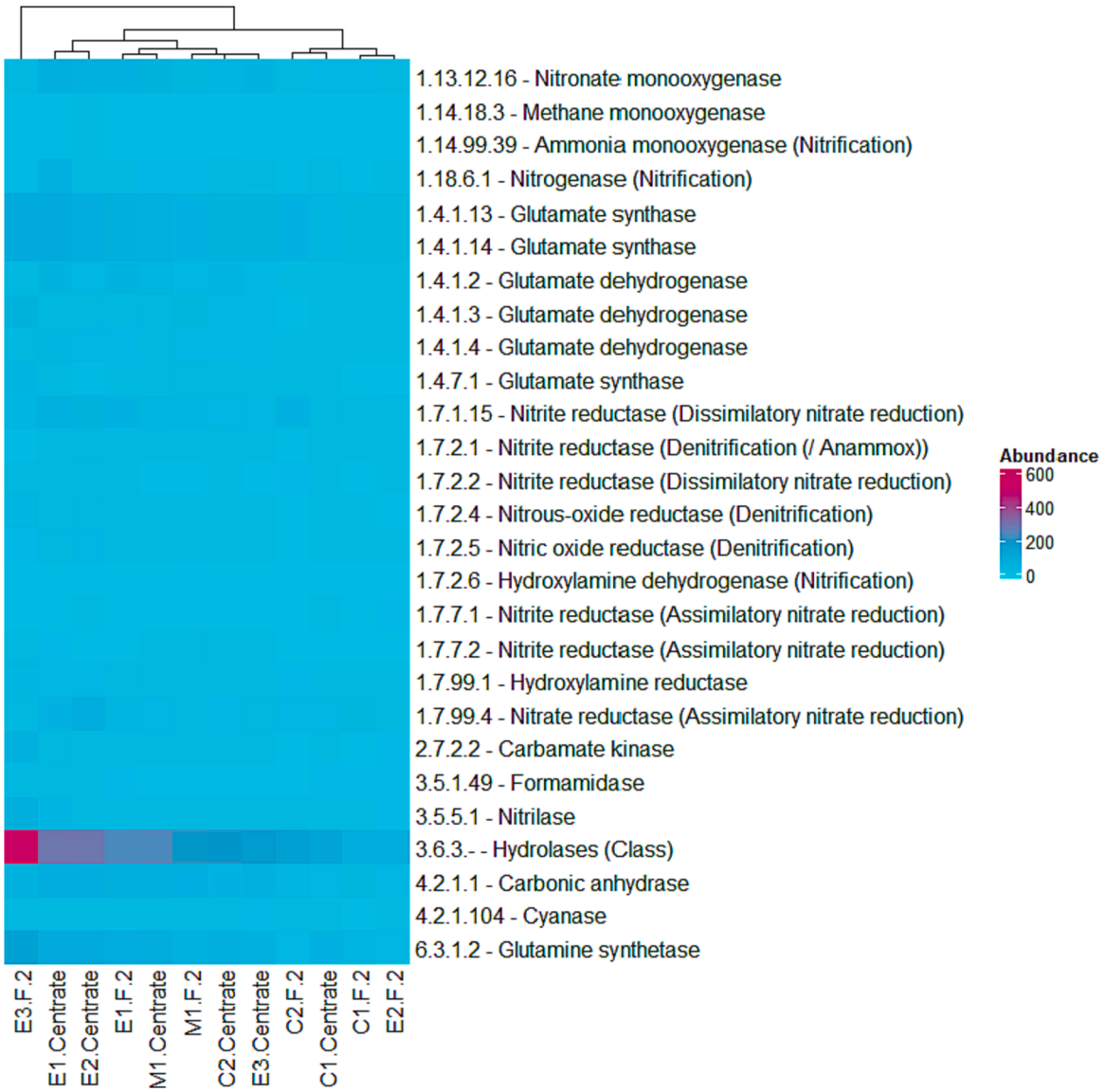


Fig. 3. Enzyme abundance profile inferred by iVikodak for the N metabolism.

quenching, co-culture with cyanobacteria, and zeaxanthin production (Zheng and Sun, 2019). On the other hand, *GpI* showed negative interaction with *Penicillium,* possibly important in terms of fungal inhibition due to metabolites (Han et al., 2021), while *GpIV* showed a positive interaction with the bacterivore *Colpoda.*

3.5. Nitrogen metabolism

The amount of supplied nitrogen was non-limiting in both media, thus it was well above the quantity of N actually included in the biomass, equal to about 6 % in the case of centrate and 13 % in the case of F/2, but the two experimental designs are distinguished mainly by the source and quantity of N present in the medium. The biomass grown on diluted centrate were exposed to a high concentration of NH_4^+ and showed a higher quantity of N included in the biomass (5.6 % dry weight, Table 2) than the biomass grown on NO_3^- (3.9 % dry weight). Therefore, different metabolic pathways might be selected for the two forms of N supplied. When looking at the prokaryotic communities, however, multiple N pathways are present (N cycle). Here, the prokaryotic pathway of the enzyme profile for N metabolism was investigated through iVikodak (Fig. 3). In general, bacterial communities cultivated in centrate showed a trend for a higher abundance of genes coding for N metabolism enzymes (except for E3), possibly due to the higher content and different forms of N present in the supplied medium when compared to F/2.

When considering the main bacterial pathways of the N cycle, all samples presented an absence of genes coding for the anammox processes and for the reduction of NO_3^- to NO_2^- through DNRA and denitrification and for the oxidation of NO_2^- to NO_3^- through nitrification. C1 cultures grown on centrate seemed to have a higher potential to carry out N fixation and assimilatory nitrate reduction (ANR) (steps from NO_2^- to NH_3) and dissimilatory nitrate reduction (DNRA) than the C1 cultures grown on F/2. C2 cultures in centrate showed again a trend of increase in the processes of ANR (steps from NO_3^- to NO_2^- and NH_3), denitrification (steps from NO_2^- to N_2O, NO and N_2) and N fixation. E1, E2 and M1 showed a higher similarity between F/2 and centrate media: E1 cultures seemed to have a lower potential, again in F/2, to carry out ANR (steps from NO_3^- to NO_2^-) while E2 showed an increase in F/2 of ANR (steps from NO_3^- to NO_2^-) but a decrease in denitrification (steps from NO to N_2). M1 showed a slightly increased ability to carry out ANR (steps from NO_2^- to NH_3) in centrate than in F/2. E3 showed an increase from centrate to F/2 in the reduction of NO_2^- to NH_3 through DNRA, while a decrease in the potential of reduction of the ability to reduce NO_2^- to N_2O and NO through denitrification and N fixation was detected.

4. Conclusions

During cultivation, eukaryotic inocula were replaced by other eukaryotic microalgae and cyanobacteria, while cyanobacterial inocula were maintained. Communities were similar for the same inoculum in both media, however, inoculation with cyanobacteria seemed to lead to higher variability within eukaryotic communities. When the community differed between media, centrate communities showed higher richness and diversity, possibly supported by the medium itself. The higher N content of centrate possibly contributed to the trend of a higher abundance of N metabolism genes. Dynamics and growth between media were comparable, showing that centrate is a viable and efficient alternative for combining biomass production and bioremediation.

Funding

This study is part of the European Union's Horizon 2020 Research and Innovation Program under Grant Agreement No. 727874 (SABANA).

CRediT authorship contribution statement

Elisa Clagnan: Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Giuliana D'Imporzano:** Conceptualization, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Marta Dell'Orto:** Investigation, Writing – review & editing. **Alessia Bani:** Methodology, Data curation, Writing – review & editing. **Alex J. Dumbrell:** Supervision. **Katia Parati:** Supervision. **Francisco Gabriel Acién-Fernández:** Conceptualization, Supervision, Funding acquisition, Project administration, Writing – review & editing. **Agustín Portillo-Hahnefeld:** Investigation. **Antera Martel-Quintana:** Investigation. **Juan Luis Gómez-Pinchetti:** Supervision, Resources, Writing – review & editing. **Fabrizio Adani:** Conceptualization, Supervision, Funding acquisition, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2022.127979.

References

Ayre, J.M., Mickan, B.S., Jenkins, S.N., Moheimani, N.R., 2021. Batch cultivation of microalgae in anaerobic digestate exhibits functional changes in bacterial communities impacting nitrogen removal and wastewater treatment. Algal Res. 57, 102338.

Bani, A., Fernandez, F.G.A., D'Imporzano, G., Parati, K., Adani, F., 2021. Influence of photobioreactor set-up on the survival of microalgae inoculum. Bioresour. Technol. 320, 124408.

Cappello, S., Denaro, R., Genovese, M., Giuliano, L., Yakimov, M.M., 2007. Predominant growth of *Alcanivorax* during experiments on "oil spill bioremediation" in mesocosms. Microbiol. Res. 162 (2), 185–190.

Dumbrell, A.J., Ferguson, R.M.W., Clark, D.R., 2016. Microbial community analysis by single-amplicon high-throughput next generation sequencing: Data analysis - From raw output to ecology. In: McGenity, T., Timmis, K., Nogales, B. (Eds.), Hydrocarbon and Lipid Microbiology Protocols. Springer Protocols Handbooks, Springer, Berlin, Heidelberg.

Gautam, K., Tripathi, J.K., Pareek, A., Sharma, D.K., 2019. Growth and secretome analysis of possible synergistic interaction between green algae and cyanobacteria. J. Biosci. Bioeng. 127 (2), 213–221.

Ge, S., Qiu, S., Tremblay, D., Viner, K., Champagne, P., Jessop, P.G., 2018. Centrate WW treatment with *Chlorella vulgaris*: Simultaneous enhancement of nutrient removal, biomass and lipid production. Chem. Eng. J. 342, 310–320.

González-Pérez, B.K., Rivas-Castillo, A.M., Valdez-Calderón, A., Gayosso-Morales, M.A., 2022. Microalgae as biostimulants: a new approach in agriculture. World J. Microbiol. Biotechnol. 38, 1–12.

Griffith, D.M., Veech, J.A., Marsh, C.J., 2016. cooccur: Probabilistic species co-occurrence analysis in R. J. Stat. Softw. Code Snippets 69 (2), 1–17.

Guillard, R.R.L., Ryther, J.H., 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea*. Cleve. Can. J. Microbiol. 8, 229–239.

Hamida, R.S., Ali, M.A., Redhwan, A., Bin-Meferij, M.M., 2020. Cyanobacteria - A promising platform in green nanotechnology: A review on nanoparticles fabrication and their prospective applications. Int. J. Nanomed. 15, 6033–6066.

Han, S., Zhou, Q., Lilje, O., Xu, W., Zhu, Y., van Ogtrop, F.F., 2021. Inhibition mechanism of *Penicillium chrysogenum* on *Microcystis aeruginosa* in aquaculture water. J. Clean. Prod. 299, 126829.

Hawrot-Paw, M., Koniuszy, A., Gałczyńska, M., Zając, G., Szyszlak-Bargłowicz, J., 2020. Production of Microalgal Biomass Using Aquaculture Wastewater as Growth Medium. Water 12, 106.

Hempel, N., Petrick, I., Behrendt, F., 2012. Biomass productivity and productivity of fatty acids and aminoacids of microalgae strains as key characteristics of sustainability for biodiesel production. J. Appl. Phycol. 24 (6), 1407–1418.

Herlemann, D.P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J.J., Andersson, A.F., 2011. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME J. 5 (10), 1571–1579.

Kadukova, J., 2016. Surface sorption and nanoparticle production as a silver detoxification mechanism of the freshwater alga *Parachlorella kessleri*. Bioresour. Technol. 216, 406–413.

Kawai, S., Murata, K., 2016. Biofuel production based on carbohydrates from both brown and red macroalgae: recent developments in key biotechnologies. Int. J. Mol. Sci. 17.

Khan, W., Rayirath, U.P., Subramanian, S., Jithesh, M.N., Rayorath, P., Hodges, D.M., Critchley, A.T., Cragie, J.S., Norrie, J., 2009. Seaweed extracts as biostimulants of plant growth and development. J. Plant Growth Regul. 28, 386–399.

Krivina, E.S., Temraleeva, A.D., 2020. Identification problems and cryptic diversity of *Chlorella*-clade microalgae (Chlorophyta). Microbiol. 89, 720–732.

Kumar, A., Bera, S., 2020. Revisiting nitrogen utilization in algae: A review on the process of regulation and assimilation. Bioresour. Technol. Rep. 12, 100584.

Ledda, C., Romero Villegas, G.I., Adani, F., Acién Fernández, F.G., Molina Grima, E., 2015. Utilization of centrate from WW treatment for the outdoor production of *Nannochloropsis gaditana* biomass at pilot-scale. Algal Res. 12, 17–25.

Liesack, W., Finster, K., 2005. *Desulfuromusa*. In: Brenner, D.J., Krieg, N.R., Staley, J.T., Garrity G.M. (eds) Bergeýs Manual of systematic bacteriology, Volume 2, The Proteobacteria, 1010-1013.

Liu, W., Jiang, J., Tan, Y., Lin, X., 2020. Novel contributions to the taxonomy of the Ciliates genus *Euplotes* (Ciliophora, Euplotida): Redescription of two poorly known species, with a brief note on the distributions of this genus in coastal waters of Southern China. Front. Mar. Sci. 7, 615413.

López-Pérez, M., Gonzaga, A., Ivanova, E.P., Rodriguez-Valera, F., 2014. Genomes of *Alteromonas australica*, a world apart. BMC Genom. 15, 483.

Lu, D., Zhang, X., Liu, X., Zhang, L., Hines, M., 2018. Sustainable microalgae cultivation by using anaerobic centrate and biogas from anaerobic digestion. Algal Res. 35, 115–124.

Lucena, T., Sanz-Sáez, I., Arahal, D.R., Acinas, S.G., Sánchez, O., Pedrós-Alió, C., Aznar, R., Pujalte, M.J., 2020. *Mesonia oceanica* sp. nov., isolated from oceans during the Tara oceans expedition, with a preference for mesopelagic waters. Int. J. Syst. Evol. Microbiol. 70, 4329–4338.

Martinez, A.P., 2020. pairwiseAdonis: Pairwise multilevel comparison using adonis. R package version 4.

Massana, R., Labarre, A., López-Escardó, D., Obiol, A., Bucchini, F., Hackl, T., Fischer, M. G., Vandepoele, K., Tikhonenkov, D.V., Husnik, F., Keeling, P.J., 2021. Gene expression during bacterivorous growth of a widespread marine heterotrophic flagellate. ISME J. 15, 154–167.

McMurdie, P.J., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8, e61217.

Medina-Cabrera, E.V., Rühmann, B., Schmid, J., Sieber, V., 2020. Characterization and comparison of *Porphyridium sordidum* and *Porphyridium purpureum* concerning growth characteristics and polysaccharide production. Algal Res. 49, 101931.

Merlo, S., Gabarrell Durany, X., Pedroso Tonon, A., Rossi, S., 2021. Marine microalgae contribution to sustainable development. Water 13 (10), 1373.

Molina-Grima, E., García-Camacho, F., Acién-Fernández, F.G., Sánchez-Mirón, A., Plouviez, M., Shene, C., Chisti, Y., 2022. Pathogens and predators impacting commercial production of microalgae and cyanobacteria. Biotechnol. Adv. 55, 107884.

Morillas-España, A., Ruiz-Nieto, Á., Lafarga, T., Acién, G., Arbib, Z., González-López, C. V., 2022. Biostimulant capacity of chlorella and chlamydopodium species produced using wastewater and centrate. Biology 11, 1086.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., et al., 2020. Vegan: Community ecology package. R package version 2.

Pahunang, R.R., Buonerba, A., Senatore, V., Oliva, G., Ouda, M., Zarra, T., Muñoz, R., Puig, S., Ballesteros, F.C., Li, C.-W., Hasan, S.W., Belgiorno, V., Naddeo, V., 2021. Advances in technological control of greenhouse gas emissions from wastewater in the context of circular economy. Sci. Total Environ. 792, 148479.

Pawar, S.T., Puranik, P.R., 2008. Screening of terrestrial and freshwater halotolerant cyanobacteria for antifungal activities. World J. Microbiol. Biotechnol. 24, 1019–1025.
Piredda, R., Tomasino, M.P., D'Erchia, A.M., Manzari, C., Pesole, G., Montresor, M., Zingone, A., 2017. Diversity and temporal patterns of planktonic protist assemblages at a mediterranean long term ecological research site. FEMS Microbiol. Ecol. 93, 1, fiw200.
Qiu, S., Yu, Z., Hu, Y., Chen, Z., Guo, J., Xia, W., Ge, S., 2021. An evolved native microalgal consortium-snow system for the bioremediation of biogas and centrate wastewater: Start-up, optimization and stabilization. Water Res. 196, 117038.
Romero-Villegas, G.I., Fiamengo, M., Acién-Fernández, F.G., Molina-Grima, E., 2018. Utilization of centrate for the outdoor production of marine microalgae at the pilot-scale in raceway photobioreactors. J. Environ. Manage. 228, 506–516.
Romero-Villegas, G.I., Burboa-Charis, V.A., Navarro-López, E., Cerón-García, M.C., Acién-Fernandez, F.G., Estrada-Alvarado, M.I., Rout, N.P., Cira-Chávez, L.A., 2021. Biomass production and urban centrate nutrient removal using native microalgae tolerant to high nitrogen concentration and temperature. J. Appl. Phycol. 33, 2921–2931.
Sepúlveda, C., Acién, F.G., Gómez, C., Jiménez-Ruíz, N., Riquelme, C., Molina-Grima, E., 2015. Utilization of centrate for the production of the marine microalgae *Nannochloropsis gaditana*. Algal Res. 9, 107–116.
Shin, H., Lee, E., Shin, J., Ko, S.-R., Oh, H.-S., Ahn, C.-Y., Oh, H.-M., Chi, B.-H., Cho, S., 2018. Elucidation of the bacterial communities associated with the harmful microalgae *Alexandrium tamarense* and *Cochlodinium polykrikoides* using nanopore sequencing. Sci. Rep. 8, 5323.
Singh, J.S., Kumar, A., Rai, A.N., Singh, D.P., 2016. Cyanobacteria: A precious bio-resource in agriculture, ecosystem, and environmental sustainability. Front. Microbiol. 7, 529.
Sullivan, B.K., Sherman, T.D., Damare, V.S., Lilje, O., Gleason, F.H., 2013. Potential roles of *Labyrinthula* spp. in global seagrass population declines. Fungal Ecol. 6, 5, 328–338.
Wagner-Döbler, I., Ballhausen, B., Berger, M., Brinkhoff, T., Buchholz, I., Bunk, B., Cypionka, H., Daniel, R., Drepper, T., Gerdts, G., Hahnke, S., Han, C., Jahn, D., Kalhoefer, D., Kiss, H., Klenk, H.-P., Kyrpides, N., Liebl, W., Liesegang, H., Meincke, L., Pati, A., Petersen, J., Piekarski, T., Pommerenke, C., Pradella, S., Pukall, R., Rabus, R., Stackebrandt, E., Thole, S., Thompson, L., Tielen, P., Tomasch, J., von Jan, M., Wanphrut, N., Wichels, A., Zech, H., Simon, M., 2010. The complete genome sequence of the algal symbiont *Dinoroseobacter shibae*: A hitchhiker's guide to life in the sea. ISME J. 4, 61–77.
Watanabe, K., Takihana, N., Aoyagi, H., Hanada, S., Watanabe, Y., Ohmura, N., Saiki, H., Tanaka, H., 2005. Symbiotic association in *Chlorella* culture. FEMS Microbiol. Ecol. 51 (2), 187–196.