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Cultivation of *Nannochloropsis oculata* in saline oil & gas wastewater supplemented with anaerobic digestion effluent as nutrient source

Aurélien Parsy[a], Cecilia Sambusiti[a,*], Patrick Baldoni-Andrey[a], Thomas Elan[b], Frédéric Périé[b]

[a] TOTAL SA, PERL – Pôle D'Etudes et de Recherche de Lacq, Pôle Economique 2, BP 47 – RD 817, 64170 Lacq, France
[b] TOTAL SA, Avenue Larribau, 64018 Pau Cedex, France

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ABSTRACT

This work investigates the feasibility of growing marine microalgae *Nannochloropsis oculata* in a mix of seawater and saline produced water obtained during oil & gas extraction, supplemented with liquid digestate from anaerobic digestion process as source of nutrients. In particular, three-stage cultures were conducted by varying the produced water loading in the culture media (from 0 up to 50% v/v), supplemented with 5% v/v of digestate and seawater. Growth parameters as well as nitrogen (ammonium) and organic carbon (expressed as chemical oxygen demand) removal efficiencies were monitored. Results revealed that *N. oculata* is perfectly able to grow in a seawater containing produced water from 10 up to 30% v/v. A slower growth was observed for 40 and 50% v/v of produced water, because of the salinity higher than 60 $g{\cdot}L^{-1}$. Maximal growth rates obtained were 0.35, 0.27 and 0.16 day^{-1}, with a maximal optical density of 6.3, 5.2 and 3.2 for 10, 20 and 30% v/v of produced water, respectively. *Nannochloropsis oculata* showed better removal efficiencies for ammonium nitrogen (around 100%) than for organic carbon (approximately 40% after one step of acclimation), regardless of the produced water loading, most chemical oxygen demand being volatilized and/or degraded by bacteria during the first two days of a culture. Regardless of the loading, > 90% of iron brought by produced water and digestate was precipitated and/or assimilated/adsorbed by *N. oculata*.

1. Introduction

The production of oil and gas leads to the generation of produced water (PW) with a current average water-to-oil ratio of 3:1 and expected to reach 12:1 by 2025 with the aging of oil reservoirs [1]. In 2003, it was estimated that 800 million m^3 of PW were discharged offshore in the world [2]. Considering Europe (Denmark, Germany, Ireland, Netherlands, Norway, Spain and United Kingdom), OSPAR Commission reports 308 million m^3 of PW were discharged in the sea in 2017 [3]. Physico-chemical properties and compositions of PW are highly dependent on the localization and the geological formation of the reservoir [4,5]. PW are composed of inorganic compounds such as salts (Total Dissolved solids (TDS) up to 300 $g{\cdot}L^{-1}$ [6]), metals (iron, barium, cadmium, lead, …), organic molecules such as aliphatic hydrocarbons, Polycyclic Aromatics Hydrocarbons, phenols, benzene, toluene, ethylbenzene, xylene (BTEX), organic acids, etc.… and sometimes naturally occurring radioactive materials [6,7]. Moreover, PW can also contain chemical additives (corrosion inhibitors, biocides, emulsion breakers, wax inhibitors, asphaltene inhibitors, H_2S scavengers, etc.) added to enhance oil extraction and to facilitate oil, gas and water separation processes [8]. The presence of all these chemicals and organic pollutants affects PW toxicity and its biodegradation in bioremediation processes [8]. Due to its environmental impact and current legislations, PW must be treated before being re-injected or discharged. Several technologies are actually used to treat dissolved organic pollutants in PW: physical and chemical processes such as coagulation, hydrocyclones, flotation units and membrane filtration [7,9] and biological treatments such as biological aerated filters [7] and membranes bioreactors [8,10].

Microalgae are photoautotrophic microorganisms able to use light to fix inorganic carbon such as CO_2. Some microalgae are able to grow without light (i.e. heterotrophic growth) and, in this case, their energy and carbon source is organic (i.e. glucose, galactose, fructose, acetate and glycerol) [11]. Other microalgae are able to grow using both

Abbreviations: BTEX, benzene, toluene, ethylbenzene and xylene; COD, chemical oxygen demand; IC, inorganic carbon; IC50, half maximal inhibitory concentration; PAR, photosynthetically active radiation; PW, produced water; STD, standard deviation; TC, total carbon; TDS, total dissolved solids; TOC, total organic carbon; TS, total solids; TSS, total suspended solids; VS, volatile solids

* Corresponding author.
E-mail address: cecilia.sambusiti@total.com (C. Sambusiti).

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photoautotrophic and heterotrophic metabolisms, and so called mixotrophic organisms [12]. These particular microalgae are able to assimilate organic molecules in addition to carbon dioxide to produce biomass, potentially reducing the amount of organic carbon (COD, TOC) in PW when it used as culture medium [12–14]. Microalgae also assimilate some metals available in PW (i.e. iron, zinc, etc.) to use them as oligo-elements for their growth [14], this underlines their potential use for the PW bioremediation. Godfrey [15] shows that various microalgae strains (i.e. *Amphora coffeaeformis*, *Chaetoceros gracilis*, *Phaeodactylum tricornutum*, DA116, BA117, BA118, BA050 and USU080) isolated from Great Salt Lake (Utah, USA) can grow in PW with low salinity (TDS = 26 $g{\cdot}L^{-1}$) complemented with nitrogen (i.e. $N\text{-}NO_3^-$) and phosphorous (i.e. $P\text{-}PO_4^{3-}$) [15].

Abdul Hakim et al. [14] screened nine strains of microalgae in PW and identified five green algae species able to grow in them (*Dictyosphaerium* sp., *Chlorella* sp., *Scenedesmus* sp., *Monoraphidium* sp. and *Neochloris* sp.). Authors have shown that these species are particularly efficient to adsorb some metals from PW, especially *Dictyosphaerium* sp. able to remove 14, 21, 20, 92, 19 and 92% of magnesium, strontium, boron, copper, chromium and nickel, respectively. Arriada and Abreu [4] showed that marine microalgae (i.e. *Nannochloropsis oculata*) used to grow in saline medium are able to grow in a mixture of PW and synthetic growth medium (F/2 medium). They conducted growth experiments with the green algae *Nannochloropsis oculata* in a mix of PW and F/2 medium (0, 50 and 100% v/v of PW, with a final salinity of 25 $g{\cdot}L^{-1}$) and obtained growth rates of 0.13, 0.12 and 0.06 day^{-1} for 0, 50 and 100% v/v of PW loading, respectively.

Better growth rates were observed at 50 and 100% v/v of PW loading (0.22 and 0.09 day^{-1}, respectively) after an acclimation phase in PW. Recently, Ammar et al. [16] conducted growth experiments with *N. oculata* using various PW loading supplemented with BG-11 medium, as nutrient source. Similar growth rates were obtained by using pure BG-11 medium (0.15 day^{-1}) and 25% v/v of PW (0.18 day^{-1}). However, it becomes more difficult to grow the microalgae at higher PW loading (at 50% v/v), resulting in a 20% decrease of growth rate (0.12 day^{-1}) compared to that obtained by using BG-11 medium alone, probably due to a high salinity (TDS of PW was 137.8 $g{\cdot}L^{-1}$). These grow rates are higher than those obtained by Arriada & Abreu [4], explained by a difference in PW composition and/or by a higher concentration of nutrients in BG-11 than in F/2 medium. Ammar et al. [16] also showed that *N. oculata* is also potentially able to remove COD from PW, with an higher removal efficiency at low PW loading (90% and 54% of COD removal for 10% v/v and 50% v/v PW loading, respectively) [16]. However, this decrease can also be due to the higher salinity at 50% v/v than at 10% v/v PW loading. Indeed, high salinity is known to reduce the bioremediation efficiency of bacteria and microalgae [17].

Anaerobic digestion is a source of renewable energy and nutrients, which can potentially be used to grow microalgae instead of commercial synthetic media [18–20]. This process involves the degradation and stabilization of organic matter under anaerobic conditions by microbial organisms and leads to the formation of biogas, mainly composed of CH_4 (55–75%), CO_2 (25–45%) and other trace elements and digestate which contains residual organic fraction and nutrients [18]. Generally, digestate is separated mechanically into liquid (80–90%) and solid fractions (10–20%), using belt press, sieve drum, screw press, sieve centrifuge, rotary screen and decanter centrifuge [18,20]. Liquid fraction is highly turbid (up to 2000 NTU) and contains large quantities of COD (up to 6.9 $g_{COD}{\cdot}L^{-1}$) and nitrogen (up to 3.4 $g_N{\cdot}L^{-1}$), mainly ammonium (65–98%), smaller amount of phosphorous (< 400 $mg_P{\cdot}L^{-1}$) and metals [18]. Considering its composition and the high turbidity, liquid digestate has to be diluted before using as culture medium, in order to prevent the inhibitory effect of ammonium present in high concentration (> 200 $mg{\cdot}L^{-1}$) [19,21,22] and to reduce turbidity to give microalgae access to light [22].

Only one study concerned the growth of microalgae in PW from gas exploitation supplemented with liquid digestate. Racharaks et al. [19] tested a mix of flowback water from shale gas exploration (water that flows back to the surface during and after the completion of hydraulic fracturing to bring the well online) and anaerobic digestion effluent as culture medium for microalgae. Four liquid digestate loadings (2, 4, 6 and 10% v/v) were tested in flowback water (TDS = 42 $g{\cdot}L^{-1}$, $N\text{-}NH_4^+$ = 75–190 $mg{\cdot}L^{-1}$, $P\text{-}PO_4^{3-}$ = 2–11 $mg{\cdot}L^{-1}$) to grow *Nannochloropsis salina*. For these conditions, growth rates were approximately 0.23 day^{-1} for 6 and 10% v/v and 0.28 day^{-1} for 2% v/v of digestate loadings. More experiments realized at 6% v/v of digestate loading and in F/2 medium, both in flowback water, showed no difference of growth parameters (growth rate and average biomass productivity) between the two media. Therefore, liquid digestate seems to be a reliable source of nutrient for growing of microalgae.

In this context, the objective of this study was to investigate the feasibility of using PW from oil extraction supplemented with liquid digestate as an innovative culture medium to grow marine green microalgae *Nannochloropsis oculata*, instead of fresh water. At the same time, this study also investigates the first step of a circular economy based on microalgae growth thanks to CO_2 capture, nutrient recycling through anaerobic digestion, PW bioremediation and renewable energy production.

2. Materials and methods

2.1. Influence of salinity on N. oculata growth

Nannochloropsis oculata (Greensea, Mèze, France) culturing tests were performed by using 500 mL glass test bottles. Light was provided with 14/10 h light/dark periods, by 3 LED lamps (CorePro LEDtube, 23 W, 2700 lm, 6500K, Philips, Netherlands), corresponding to a photosynthetically active radiation (PAR) of 50, 100 and 150 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ when 1, 2 or 3 lamps were used, respectively. Light was increased by step during the culture according to cultures optical densities, to 100 and 150 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ when majority of cultures reached an optical density of 0.9 and 1.5, respectively. Microalgae grew at (21 ± 1 °C), under autotrophic conditions with air and CO_2 bubbling at the bottom of the bottles. CO_2 was added in pulse, 5 s each 20–40 min and manually adjusted to maintain pH between 7.5 and 9.

Tests were conducted in sterile commercial F/2 medium [23] at various salinities (30, 50, 60, 70, 80, 90 and 100 $g{\cdot}L^{-1}$), by using Instant Ocean salts (Aquarium Systems, France) in order to study the influence of salinity on *Nannochloropsis oculata* growth and determine the half maximal inhibitory concentration of salts. These experiments were realized in duplicate for 22 days.

2.2. Nannochloropsis oculata inoculum

To start experiments with the mix of PW, liquid digestate and seawater, an inoculum was prepared by growing *Nannochloropsis oculata* in F medium (doubled concentrations of F/2 medium) at 50 $g{\cdot}L^{-1}$ of salinity at the same conditions as described above. Once microalgae reached the stationary phase at enough concentration (at least an optical density of 4, corresponding to approximately 0.7 $g_{TSS}{\cdot}L^{-1}$), they were inoculated in 500 mL glass bottles (10% v/v of the final volume) to start culture tests with a medium composed of a mix of PW, liquid digestate and supplemented with seawater.

2.3. Culture tests in real produced water and liquid digestate

Three successive stages of culture (i.e. called stage 1, stage 2 and stage 3) were realized to evaluate the growth parameters of *N. oculata* in different proportions of PW, as shown in Fig. 1.

The first stage was conducted to study the feasibility of using PW as culture medium in addition to liquid digestate and to determine *N. oculata* growth parameters.

During the 1st stage, tests were performed with a mix containing microalgae from the preliminary culture (10% v/v), real PW from a

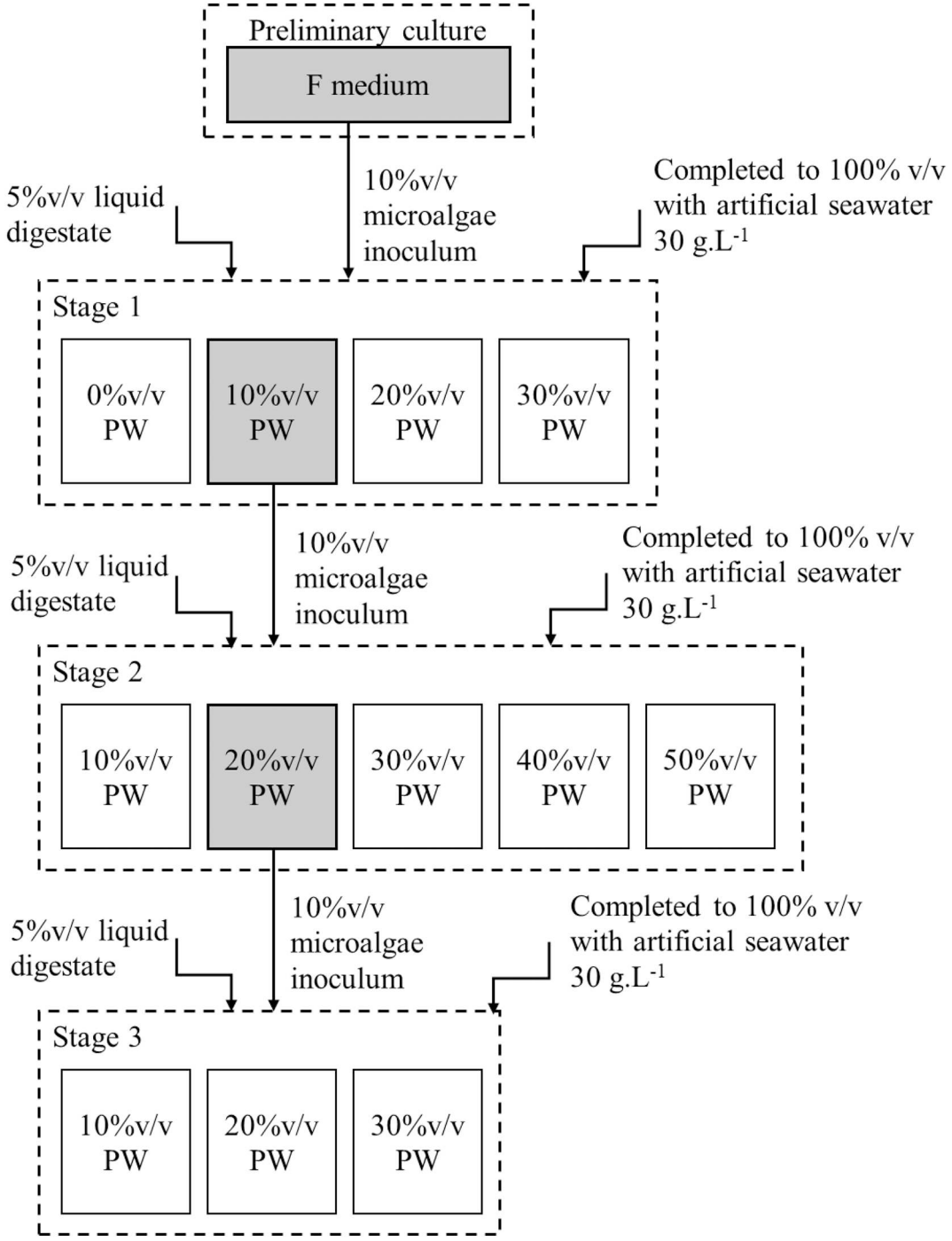

Fig. 1. Experimental plan of *Nannochloropsis oculata* growth in produced water (PW) and liquid digestate.

TOTAL operating site (from 0 to 30% v/v), liquid digestate from an industrial scale biogas plant (Aire-sur-Adour, France) filtered on 50 μm filter (5% v/v) and artificial seawater at 30 $g{\cdot}L^{-1}$ (Instant Ocean salts, Aquarium Systems, France) to complete the final volume.

During the 2nd and 3rd stages, culture with increasing PW loadings were made to evaluate the potential acclimation of *N. oculata* to PW and determine if its kinetic growth parameters might be improved. All tests were performed in duplicate. Each stage of culture was realized for 23–29 days, until the stationary phase was reached.

In a culture medium composed of a mix of PW and liquid digestate, microalgae can grow under mixotrophic conditions: organic carbon is provided by PW and digestate, and inorganic carbon is provided by CO_2 injections. Microalgae grew in the same conditions of pH, temperature and light as described in 2.1. Nitrogen (mostly $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$) and phosphorous ($P\text{-}PO_4^{3-}$) sources were supplemented by adding 5% v/v of filtered digestate in each reactor for all batches. Moreover, phosphate concentrations were fixed with $NaH_2PO_4\text{-}H_2O$ powder to obtain 5 $mg{\cdot}L^{-1}$ in each reactor in order to maintain a N:P ratio around 16:1, depending of the concentration of ammonium at each batch. Salinity varied between 33 and 74 $g{\cdot}L^{-1}$ and its concentration was strictly dependent on the PW loading in each bottle test.

2.4. Analytical methods

2.4.1. Physico-chemical composition of produced water and liquid digestate

PW and liquid digestate were characterized in terms of physico-

chemical composition. pH was measured by using pH-meter HQ40d (Hach company, Loveland, Colorado, USA) and K912 conductivity meter (Consort, Belgium) was used to monitored conductivity. Salinity was estimated from the conductivity and was expressed in $g_{NaCl}{\cdot}L^{-1}$. Turbidity and optical density at 680 nm (OD) were measured by a 2100Qis portable turbidimeter (Hach company, Loveland, Colorado, USA) and a UV–Vis spectrophotometer (Thermoscientific Evolution 201 UV–visible, USA) respectively. Heavy metals were measured by inductively coupled plasma atomic emission spectroscopy (ICP/AES) for B, Ca, Fe, Mg, inductively coupled plasma mass spectrometry (ICP/MS) for K, Al, As, Ba, Be, Cd, Cr, Co, Cu, Mo, Ni, Pb, V, Zn and by atomic fluorescence spectrometry (for Hg) according to standard methods [24–26]. Organic compounds such as Naphthalene, benzene, toluene, ethylbenzene and xylene were analyzed by gas chromatography coupled to mass spectrometry (GC/MS). Volatile fatty acids (VFA) present in liquid digestate were analyzed after 0.2 µm filtration, using a gas chromatograph (GC-7090 B, Agilent, USA) equipped with a CP 8400 sampler, a FFAP ECTM 1000 column and with a flame ionization detector (FID). Acetic (C2), propionic (C3), butyric and iso-butyric (C4 and iC4), valeric and iso-valeric (C5 and iC5) and caproic (C6) acids standards were purchased from Sigma Aldrich (USA). Permeates were used to monitor nutrients and dissolved organic/inorganic carbon, after a consecutive filtration using glass fiber filters of 0.8 and 0.2 µm. Total carbon (TC), inorganic carbon (IC) and total organic carbon (TOC) were analyzed by a TOC-meter (Shimadzu TOC-L, Japan). Chemical oxygen demand (COD) and ammonium nitrogen (NH_4^+) concentrations were evaluated by spectrophotometric methods with LCK_{1414} kit (for COD) and $LCK_{303,\ 304,\ 305}$ kits (for NH_4^+) (Hach Company, Loveland, Colorado, USA). Nitrate (NO_3^-) and phosphate (PO_4^{3-}) concentrations were measured by Ionic liquid chromatography (Dionex ICS1000, column AS9-HC, 4 ∗ 250 mm, Thermoelectro, USA).

2.4.2. Chemical composition of Nannochloropsis oculata

Analysis was made to characterize in terms of chemical composition the microalgal strain (Greensea, France). For this purpose, samples were centrifuged three times at 4400*g* and the pellet was rinsed three times with milli-Q water. Then, microalgae were dried 4 days at 40 °C. Total solids (TS), volatile solids (VS) and Ash were analyzed according to standard methods [27].

C, H, N, S, O analysis (FlashSmart Elemental Analyser, ThermoFisher, USA) was performed on dried samples to measure the percentage of carbon, hydrogen, nitrogen, sulfur and oxygen in microalgal cells. Protein content was then estimated by multiplying N content by 4.78 [28].

Carbohydrates content was evaluated by acid hydrolysis followed by a High-performance liquid chromatography method. Carbohydrates were first extracted from microalgae and fragmented by a two-step acid hydrolysis. Briefly, 1 mL of H_2SO_4 (72%) was added to 80 mg of microalgae powder and incubated 30 min at room temperature. Then, 11 mL of deionized water was added, and samples were incubated 2 h at 100 °C under stirring (350 rpm). After incubation, samples were filtered with 0.2 µm filter. Finally, soluble sugars (i.e. glucose, xylose, arabinose) were analyzed by high-pressure liquid chromatography (HPLC) system (Agilent Technologies, USA), equipped with an Aminex HPX-87H (Biorad, Marnes-la-Coquette, France) column at 40 °C, a refractive index detector at 40 °C and a 0.005 $mol{\cdot}L^{-1}$ H_2SO_4 solvent at 0.3 $mL{\cdot}min^{-1}$. Glucose, xylose and arabinose standards were provided by Sigma Aldrich (USA). Lipid content was estimated by difference and was calculated with Eq. (1):

$$100 - (\text{weight in grams [proteins+carbohydrates + ash] in 100 g of dried weight}) \quad (1)$$

2.4.3. Monitoring of the microalgae growth

Samples were collected two or three times per week. Parameters monitored during culture tests were optical density, cell count (Malassez cell counting chamber, Herka, France), pH, conductivity, COD, and NH_4^+ according to methods described in Section 2.4.1. COD and NH_4^+ were monitored during stage 1 and 2 (Fig. 1) to determine the potential bioremediation of PW by microalgae. COD and NH_4^+ removal were calculated as decrease of concentration relative to their initial values and expressed in %.

2.4.4. Assimilation/adsorption of metals in Nannochloropsis oculata

At the end of stage 1, microalgae and culture medium were separated by centrifugation at 17,200*g*. Metals content was determined on both supernatants and residues after centrifugation in order to verify the mass balance and evaluate the assimilation/adsorption rate of metals by *Nannochloropsis oculata*. Residues were rinsed with deionized water after a two-step centrifugation at 17,200*g*. Then, they were dried for 4 days at 40 °C. Metals content was obtained according to methods described in Section 2.4.1. For microalgae powder, aqua regia extractions were performed before analysis according to standard methods [29].

2.5. Data processing and statistical analysis

To fit optical density data and calculate maximal growth rates and lag phase durations, the re-parameterisation of Logistic function proposed by Zwietering et al. [30] was used:

$$OD(t) = \frac{A}{1 + \exp\left[\frac{4\mu}{A}(\lambda - t) + 2\right]} \quad (2)$$

where:

µ is the maximal specific growth rate.
λ is the lag phase duration.
A is the asymptote reached in stationary phase.

Then, doubling time T_G was determined using:

$$T_G = \frac{\ln(2)}{\mu} \quad (3)$$

Data fitting was realized using the solver of Microsoft Excel (Microsoft, USA) with a determination coefficient higher than 0.977 for all data analyzed.

To determine the half maximal inhibitory concentration (IC50) of salts, a four-parameter dose-response curve was fitted with final OD values measured during batch at various salinities (30 to 100 $g{\cdot}L^{-1}$) using GraphPad Prism (GraphPad Software, USA):

$$\%\text{Inhibition(C)} = \text{Bottom} + \frac{(\text{Top}-\text{Bottom})}{(1 + 10\text{^}((\text{LogIC50}-\text{LogC})*\text{HillSlope}))} \quad (4)$$

where:

IC50 is the half maximal inhibitory concentration
Top and Bottom are plateaus
HillSlope describes the steepness of the family of curves.

A *t*-test for paired data was performed to evaluate differences in maximal cell concentration and biomass concentration (analyzed as optical density at 680 nm) between the test conditions. A confidence level of 95% (significance α level of 0.05) was considered. Thus, *p*-values < 0.05 were deemed to be statistically significant.

3. Results and discussion

3.1. Characterization of Nannochloropsis oculata

Nannochloropsis oculata was characterized before culture experiments in terms of TS, VS, ash, proteins, carbohydrates, lipids

Table 1
Chemical composition of *Nannochloropsis oculata*. Values correspond to mean ± standard deviation (STD) of measurement performed in duplicate. TS: total solids; VS: volatile solids.

	Mean ± STD
TS (g/100 $g_{Fresh\ Matter}$)	96.0 ± 0.2
VS (g/100 gTS)	92.8 ± 0.2
Ash (g/100 gTS)	6.9 ± 0.2
C (g/100 gTS)	53.8 ± 0.3
H (g/100 gTS)	8.2 ± 0.2
N (g/100 gTS)	7.6 ± 0.2
S (g/100 gTS)	0.8 ± 0.2
O (g/100 gTS)	25.8 ± 0.2
Proteins (g/100 gTS)	36 ± 1
Carbohydrates (g/100 gTS)	7.3 ± 0.1
Glucose (g/100 gTS)	5.5 ± 2.3
Xylose (g/100 gTS)	1.8 ± 0.0
Arabinose (g/100 gTS)	0.03 ± 0.0
Lipids (g/100 gTS)[a]	49.8

[a] Lipid content was estimated by difference according to Eq. (1).

and elemental composition (C, H, N, S, O), results are shown in Table 1.

It is known that the chemical composition of microalgae (i.e. proteins, lipids and carbohydrates content) is strictly dependent on the growth conditions (i.e. nutrient concentration, salinity of the medium, light intensity and photoperiod, availability and type of carbon source). Therefore, a comparison of our results with literature data remains difficult. However, proteins content was estimated at 36%, which is coherent with values found in the literature. Na Gu et al. [31] and Paes et al. [32] reported protein contents between 30 and 35% and between 26 and 31% of dry mass respectively, for *Nannochloropsis oculata* grown in marine water. Carbohydrates were estimated at 7.3% of dry mass, which was much lower than values reported by Paes et al. [32] (from 23 to 29%), probably due to different methods of quantification. Lipids content determined in this study is coherent with values reported in literature by Wang et al. [33]; Ma et al. [34] and Nobre et al. [35] for *Nannochloropsis* sp. (37–60% w/w dry basis). These results show that *Nannochloropsis oculata* is a promising source of lipids, as it is reported in literature [31–35]. Finally, among carbohydrates, being the primary constituent of the cellulosic cell wall [36,37], glucose was the most abundant (75% of total carbohydrates).

3.2. Chemical composition of produced water and liquid digestate

Physico-chemical composition of PW and liquid digestate are reported in Table 2.

PW was slightly turbid (< 100 NTU) and characterized by a relatively high salinity (113.6 ± 11.9 $g{\cdot}L^{-1}$) and a slightly acid pH (i.e. pH = 6), COD and TOC were 562 ± 58 $mg{\cdot}L^{-1}$ and 183 ± 8 $mg{\cdot}L^{-1}$. Results were coherent with Lusinier et al. [38] who reported amount of COD in PW from 124 to 2375 $mg{\cdot}L^{-1}$ and TOC from 38 to 571 $mg{\cdot}L^{-1}$. In terms of nutrients, PW was composed of a small amount of NH_4^+ (134 $mg{\cdot}L^{-1}$) and did not contain neither NO_3^- nor PO_4^{3-}. BTEX and phenol represented < 2% of the COD. PW contained large amount of calcium and magnesium, 6.7 and 1.4 $g{\cdot}L^{-1}$ respectively. As reported by Lusinier et al. [38], PW did not contain high concentrations of heavy metals (< 0.05 $mg{\cdot}L^{-1}$ for all others), except for aluminum (0.28 $mg{\cdot}L^{-1}$), iron (23.7 $mg{\cdot}L^{-1}$) and barium (170 $mg{\cdot}L^{-1}$). Liquid digestate had a variable composition, with a turbidity of 422 ± 73 NTU, large amount of COD and NH_4^+ (1432 ± 377 $mg{\cdot}L^{-1}$ and 1561 ± 251 $mg{\cdot}L^{-1}$ respectively) and small amount of PO_4^{3-} (21.2 ± 1.1 $mg{\cdot}L^{-1}$, not detected during stage 3). TOC concentrations were highly variable during the 3 stages, with 1329, 630 and 1624 $mg{\cdot}L^{-1}$ for stage 1, 2 and 3, respectively. Thereby, organic carbon seemed less available for microalgae culture during stage 2 than during stages 1 and 3.

The composition of digestate was similar to that described by Racharaks et al. [19] in terms of salinity, pH, NH_4^+ and NO_3^-, except for PO_4^{3-} concentration, lower compared to values found in literature (i.e. 106 $mg{\cdot}L^{-1}$). Lower COD concentrations were also observed, compared to literature data (concentration range from 4 to 90 $g_{COD}{\cdot}L^{-1}$) [39], probably due to the filtration of liquid digestate (50 µm filter) applied before culture tests. Only 5% v/v of digestate loading was used to limit turbidity and NH_4^+ concentration, in order to avoid microalgae inhibition due to free ammonia. PO_4^{3-} not being detected or in low concentration, it was fixed manually to 5 $mg{\cdot}L^{-1}$ to avoid the limitation of phosphorus source for microalgae growth. VFA represented approximately 12% of total COD of digestate. Concerning metals, digestate brought trace of all metals measured, with higher concentration for calcium and iron (21.2 and 1.37 $mg{\cdot}L^{-1}$ respectively) and < 1 $mg{\cdot}L^{-1}$ for the others. Comparison of oligoelement content in PW/digestate and in F culture media (used during *N. oculata* preliminary culture) show similar order of magnitude for iron, copper and zinc concentrations, and limitation for cobalt and molybdenum in PW/digestate. Except for phosphorous, culture medium composed of a mix of PW and digestate brought organic carbon, nitrogen and mostly all trace elements necessary to microalgae growth.

3.3. Influence of salinity on Nannochloropsis oculata growth in F/2 medium

In order to find the optimal salinity condition for growing *Nannochloropsis oculata*, preliminary culture experiments were made in sterile F/2 medium by varying salinities from 30 up to 100 $g{\cdot}L^{-1}$. Fig. 2 shows the evolution of optical densities monitored during experiments.

Low or no growth were observed at salinity conditions of 70, 80, 90 and 100 $g{\cdot}L^{-1}$, preventing modeling using logistic function. At salinity conditions of 30, 50, 60 and 70 $g{\cdot}L^{-1}$, microalgal growth occurred. However, growth was strongly impacted, as final microalgae concentration and growth rates decreased with an increase of salinity. In fact, despite each batch began with the same optical density (0.25 ± 0.01), lower final values were observed with saltier medium (3.2, 2.8, 2.2 and 0.8 for 30, 50, 60 and 70 $g{\cdot}L^{-1}$ conditions respectively). Moreover, growth rates followed the same trend and decreased with an increase of salinities (0.32, 0.23 and 0.17 day^{-1} for 30, 50 and 60 $g{\cdot}L^{-1}$ conditions, respectively). Lag phases were also longer at higher salinities with 1.5, 2.8, 2.8 and 15 days for 30, 50, 60 and 70 $g{\cdot}L^{-1}$ conditions, respectively. Despite longer lag phase, exponential phases lasted 6–7 days for all conditions except 70 $g{\cdot}L^{-1}$ were it lasted 2 days.

Fitting these results with a four-parameter dose-response curve, an IC50 for salts of 63.7 $g{\cdot}L^{-1}$ (i.e. 39% v/v of PW loading) seemed to be the maximal salinity to grow *Nannochloropsis oculata* and obtain a final microalgal concentration approximately 10 times higher than the initial one.

3.4. Comparison between F/2 medium and liquid digestate as a culture medium for microalgae

Preliminary tests were performed to evaluate the feasibility to use diluted liquid digestate as a culture medium for *Nannochloropsis oculata* and in order to compare the performances of the two media (i.e. commercial F/2 and diluted liquid digestate). The trend of culture growth of *Nannochloropsis oculata* in commercial synthetic F/2 medium and in a mix of 5% v/v of liquid digestate, supplemented with seawater (both at salinity = 31 ± 1.2 $g{\cdot}L^{-1}$) is reported in Fig. 3. Temperature remained between 20 and 25 °C. Thanks to regular CO_2 injections, pH remained constant at 8.1 ± 0.5 and 7.6 ± 0.5 for F/2 medium and digestate, respectively. Light intensity was increased by step from 50 to 100 and 150 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ after 6 and 8 days, respectively, according to the optical densities of the cultures.

For both tests, initial OD_{680} was 0.31 ± 0.00. Lag phase lasted 1.5 and 3 days and exponential phase lasted 6 and 8 days by using F/2

Table 2
Chemical composition of produced water (PW) and liquid digestate used (n.d.: not determined). Values correspond to mean ± standard deviation of measurement performed in duplicate. OD_{680}: optical density at 680 nm; TC: total carbon; IC: inorganic carbon; TOC: total organic carbon; COD: chemical oxygen demand.

		PW	Liquid digestate		
			Stage 1	Stage 2	Stage 3
Turbidity	NTU	42 ± 28	502 ± 14	404 ± 7	360 ± 40
OD_{680}	–	0.12 ± 0.04	0.88 ± 0.04	0.68 ± 0.05	0.66 ± 0.01
pH	–	6.0 ± 0.2	8.3 ± 0.0	8.1 ± 0.0	7.8 ± 0.0
Conductivity	$mS{\cdot}cm^{-1}$	135.7 ± 10.9	12.4 ± 1.1	13.2 ± 0.0	13.8 ± 0.0
Salinity	$g_{NaCl}{\cdot}L^{-1}$	113.6 ± 11.9	7.3 ± 0.6	7.8 ± 0.0	8.2 ± 0.0
TC	$mg{\cdot}L^{-1}$	207 ± 12	1440 ± 24	1549 ± 43	2171 ± 7
IC		25 ± 6	111 ± 7	890 ± 12	548 ± 99
TOC		183 ± 8	1329 ± 31	630 ± 93	1624 ± 92
COD		562 ± 58	1185 ± 1	1245 ± 75	1866 ± 47
NH_4^+		134 ± 17	1665 ± 9	1275 ± 14	1743 ± 5
NO_3^-		0.0 ± 0.0	1.3 ± 0.1	0.6 ± 0.4	0.0 ± 0.0
PO_4^{3-}		0.0 ± 0.0	20.2 ± 0.3	22.2 ± 1.1	0.0 ± 0.0
Lithium (Li)		7.01	0.036	n.d.	n.d.
Boron (B)		36.1	0.22	n.d.	n.d.
Calcium (Ca)		6710	< 100	n.d.	n.d.
Iron (Fe)		23.7	1.37	n.d.	n.d.
Magnesium (Mg)		1430	21.2	n.d.	n.d.
Potassium (K)		692	n.d.	n.d.	n.d.
Aluminum (Al)		0.28	0.58	n.d.	n.d.
Arsenic (As)		< 0.05	0.021	n.d.	n.d.
Barium (Ba)		170	0.058	n.d.	n.d.
Beryllium (Be)		< 0.05	< 0.005	n.d.	n.d.
Cadmium (Cd)		< 0.002	< 0.0002	n.d.	n.d.
Chrome (Cr)		0.012	0.0041	n.d.	n.d.
Cobalt (Co)		< 0.01	0.0079	n.d.	n.d.
Copper (Cu)		0.013	0.053	n.d.	n.d.
Mercury (Hg)		< 0.000015	0.000061	n.d.	n.d.
Molybdenum (Mo)		< 0.01	0.0082	n.d.	n.d.
Nickel (Ni)		0.01	0.053	n.d.	n.d.
Lead (Pb)		< 0.01	0.0014	n.d.	n.d.
Vanadium (V)		< 0.01	0.0041	n.d.	n.d.
Zinc (Zn)		< 1	0.2	n.d.	n.d.
Benzene		0.60	n.d.	n.d.	n.d.
Toluene		0.39	n.d.	n.d.	n.d.
Ethylbenzene		0.0279	n.d.	n.d.	n.d.
o-Xylene		0.0882	n.d.	n.d.	n.d.
Naphthalene		< 0.00014	n.d.	n.d.	n.d.
Phenol index		1.7	n.d.	n.d.	n.d.
Total hydrocarbons		0.86	n.d.	n.d.	n.d.
Volatile fatty acids	$mg_{COD}{\cdot}L^{-1}$	n.d.	129	n.d.	n.d.

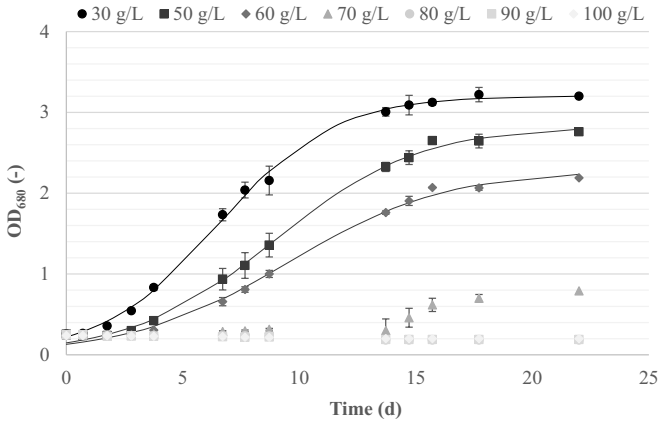

Fig. 2. Evolution of optical densities at 680 nm (OD_{680}) during culture tests at different salinities in F/2 medium. Points correspond to experimental data, lines correspond to growth modeling using the modified Logistic function. Values correspond to mean ± standard deviation of measurement performed in duplicate.

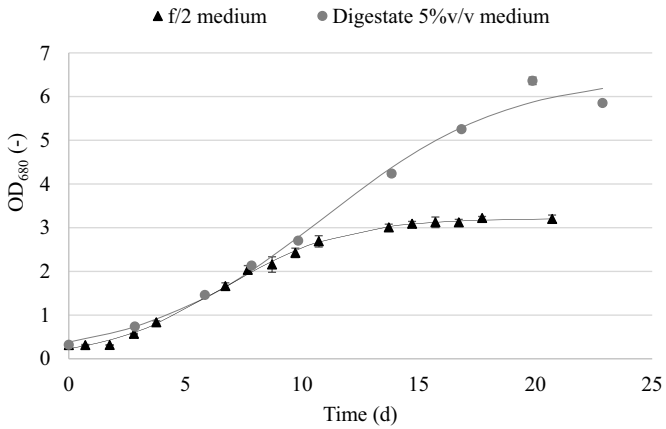

Fig. 3. Evolution of optical density at 680 nm (OD_{680}) during culture in F/2 medium and liquid digestate. Points correspond to experimental data, lines correspond to growth modeling using the modified Logistic function. Values correspond to mean ± standard deviation of measurement performed in duplicate.

medium and liquid digestate, respectively, and cultures reached the stationary phase after 15 and 20 days respectively with a maximal optical density of 3.15 ± 0.05 and 6.31 ± 0.05. Maximal growth rate reached during the exponential phase was higher with 5% v/v digestate loading (0.41 day^{-1}) than with F/2 medium (0.31 day^{-1}), corresponding to doubling times of 1.7 and 2.2 days, respectively. Lag phase twice as long with digestate might be explained by the change of nitrogen source from F/2 medium (NO_3^-) and liquid digestate ($N\text{-}NH_4^+$)

requiring to microalgae a time to adapt to this new source. It might also be explained by the competition of microalgae with other microorganisms (i.e. bacteria, fungi, protozoa) in digestate for the same nutrient source. Concerning the difference in growth kinetics and final biomass concentrations, it can be supposed that F/2 medium is more limiting as less nitrogen was available than in digestate, with 12.4 and 60.7 $mg_N \cdot L^{-1}$, respectively. Moreover, digestate also contains dissolved organic carbon that can be used by microalgae in mixotrophic metabolism, leading to a higher growth. By considering the high variability of chemical composition of digestate, results seemed in agreement with Cai et al. [40] that reported a growth rate of 0.65 day^{-1} for *Nannochloropsis* sp. growth in liquid digestate with higher light (200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 24/0 h light/dark periods). It is interesting to note that liquid digestate as culture medium permitted to obtain two times higher final biomass concentration (i.e. OD_{680} of 6) than F/2 medium (i.e. OD_{680} of 3). Thus, considering these results, diluted liquid digestate can be used to replace F/2 medium for mixotrophic growth of microalgae but a supplement in $P\text{-}PO_4^{3-}$ source is still necessary.

3.5. Feasibility and impact of produced water on Nannochloropsis oculata growth

In order to evaluate the feasibility of using PW supplemented with 5% v/v of liquid digestate and sea water for microalgae growth, experiments with different PW loading (from 0 to 30% v/v) were performed (stage 1, Fig. 1).

During these tests, temperature remained constant at 23 ± 1 °C. Thanks to regular CO_2 injections, pH remained at 7.9 ± 0.6. Light intensity was increased by step from 50 to 100 and 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ after 6 and 10 days, respectively, according to optical densities of the cultures. Fig. 4 shows the growth curve of *Nannochloropsis oculata* during stage 1 cultures. All tests began with the same optical density 0.37 ± 0.04 (1.7 ± 0.3 ∗ 10^7 cells·mL^{-1}). Results about growth parameters for the different test conditions are summarized in Table 3.

While no lag phase was observed for condition of 30% v/v PW, 3.1, 1.3 and 0.2 days of lag phase were monitored for condition of 0, 10 and 20% v/v PW, and, as expected, the higher the PW loading, the lower the final concentration of microalgae. Lower growth rates were also observed for higher PW loading, probably due to the presence of inhibitory compounds in PW (i.e. BTEX, hydrocarbons, metals) and/or by the increase in salinity which prevent an optimal growth of microalgae. Growth rate obtained during stage 1 tests were slightly higher but coherent with growth rate determined by Ammar et al. [16] in PW complemented with BG-11 medium (0.15 and 0.18 day^{-1} for 10 and 25% v/v of PW).

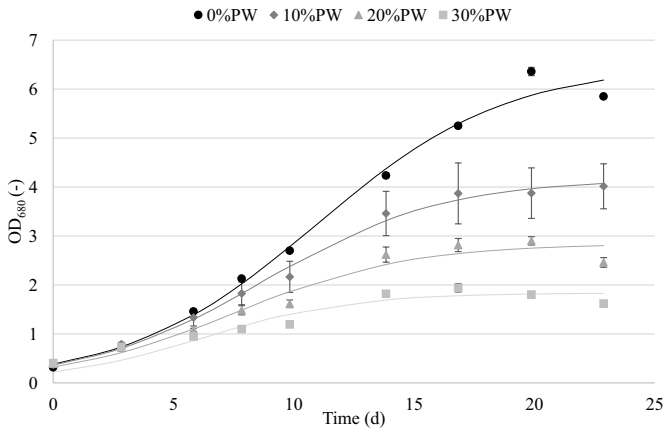

Fig. 4. Evolution of optical density at 680 nm (OD_{680}) during cultivation experiments of stage 1 at different produced water (PW) loading. Points correspond to experimental data, lines correspond to growth modeling using the modified Logistic function. Values correspond to mean ± standard deviation of measurement performed in duplicate.

To check the consistency of optical density and cell count measurements, a linear regression model apply to data was performed. It is presented in Fig. 5.

Linear correlation between optical density and cellular concentration is clearly highlighted (i.e. R^2 of 0.90). This linear model was used in order to estimate final cellular concentrations during stage 2 and 3:

$$\text{Cell concentration (expressed in cells} \cdot mL^{-1}) = OD_{680} * 2 * 10^7 + 6 * 10^6 \quad (5)$$

3.6. Acclimation of Nannochloropsis oculata to produced water

In order to verify the acclimation of *Nannochloropsis oculata* to PW, further culture experiments were made by using a microalgal inoculum previously cultivated on 10% v/v of PW, supplemented with 5% v/v of liquid digestate and sea water (condition of stage 1). The media tested in stage 2 were 10, 20, 30, 40 and 50% v/v of PW loading complemented with 5% v/v of liquid digestate to observe growth at higher PW loading and higher salinity than in stage 1 (Fig. 1). Temperature remained constant at 24 ± 1 °C. Thanks to regular CO_2 injections, pH remained at 7.9 ± 0.5. However, more frequent injections were needed to maintain pH values around 8, compared to stage 1. Light intensity was increased by step from 50 to 100 and 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ after 6 and 13 days, respectively, according to optical densities of the cultures. Fig. 6 shows the evolution of optical densities monitored during stage 2.

Each batch started with an optical density of 0.66 ± 0.05. Due to insufficient growth in condition 40 and 50% v/v PW, logistic function cannot be used to estimate growth rate and lag phase duration. Lag phase of 1.7 days was observed for 10% v/v condition while none was observed for condition 20 and 30% v/v PW. Lag phases of 2 and 14 days were manually estimated for 40 and 50% v/v of PW loading conditions, respectively, no growth being observed before these days. Due to shorter exponential phase and longer linear phase (probably due to a different composition of medium), growth parameters calculated were lower than in stage 1. Despite this, significantly higher maximal optical densities were monitored than during in stage 1 for common PW loading (p-value < 0.05). Data are summarized in Table 4.

Higher final concentrations might be explained by the higher initial biomass concentration and longer time of growth, stage 2 batches having lasted 22 (for 10 and 20% v/v of PW) to 28 days (for 30, 40 and 50% v/v of PW), 5 to 11 more days than stage 1. Doubling time determined for these three common conditions were approximately 20% higher in stage 1 than in stage 2, possibly due to a change in PW composition such as oxidation of some compounds (a color change was observed between the different stages) or a change/adaptation of bacterial population which impacted microalgal growth. As expected, almost no growth was detected for 40 and 50% v/v of PW loadings, probably due to too high salinities, 65.0 and 73.9 $g \cdot L^{-1}$ for 40 and 50% v/v of PW loadings respectively, these being definitely too high value for growth of *Nannochloropsis oculata* as discussed in Section 3.3.

Acclimation not being clearly highlighted with these experiments, stage 3 was performed by using a microalgal inoculum previously cultivated on 20% v/v of PW, supplemented with 5% v/v of liquid digestate and sea water (condition of stage 2). Conditions tested in stage 3 were 10, 20 and 30% v/v of PW loading supplemented with 5% v/v of liquid digestate (Fig. 1). The conditions with 40 and 50% v/v of PW loading were not tested again because of the excessively high salinity which inhibits microalgal growth, as described previously. Temperature remained constant between at 22 ± 1 °C. Thanks to regular CO_2 injections, pH during the different batches remained at 7.5 ± 0.6. Light intensity was increased by step from 50 to 100 and 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ after 4 and 8 days, respectively, according to optical densities of the cultures. Fig. 7 shows the evolution of optical densities monitored during stage 3.

Each batch began with an optical density of 0.46 ± 0.02. Culture with 10, 20 and 30% v/v of PW loading began with a lag phase of 2.7,

Table 3
Conditions and growth parameters of *Nannochloropsis oculata* calculated during stage 1. Values correspond to mean ± standard deviation of measurement performed in duplicate. PW: produced water; OD: optical density.

PW loading (%)	Salinity ($g \cdot L^{-1}$)	Maximal OD_{680}	Time until maximal OD (days)	Maximal cell concentration (10^7 cells·mL^{-1})	Growth rate (day^{-1})	Doubling time (days)
0	32.5 ± 0.2	6.36 ± 0.08	20	14.3 ± 1.3	0.41 ± 0.01	1.7 ± 0.0
10	39.4 ± 0.2	4.02 ± 0.46	17	8.1 ± 0.1	0.28 ± 0.05	2.5 ± 0.5
20	45.8 ± 0.0	2.90 ± 0.08	17	6.1 ± 0.1	0.20 ± 0.02	3.6 ± 0.3
30	53.0 ± 0.0	1.94 ± 0.08	17	3.2 ± 0.1	0.15 ± 0.00	4.7 ± 0.1

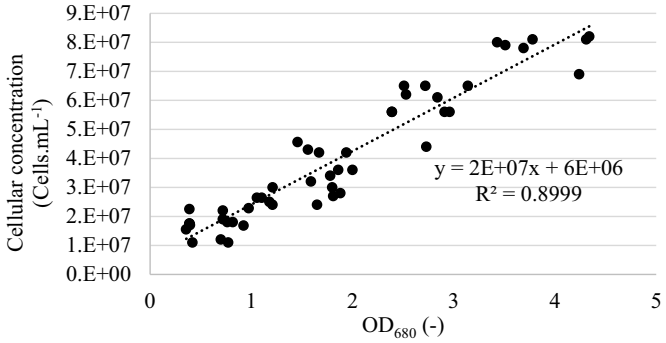

Fig. 5. Linear regression model apply to optical density (OD_{680}) and cell count data.

1.7 and 0.4 days, respectively. Exponential phase lasted 12 days for 10% v/v of PW loading and 8 days for both 20 and 30% v/v of PW loadings before microalgae go in linear then deceleration phase (Table 5).

Maximal optical densities were significantly higher than during stage 1 and 2 (p-values < 0.05). Growth rate measured during this stage were slightly higher than those obtained during stage 1 (20% higher for 10 and 20% v/v PW, 7% higher for 30% v/v of PW). Better grow rates observed during stage 3, compared to stage 1 and 2, seems to show that the acclimation of microalgae on real culture medium seems to positively influence their growth kinetics.

Fig. 8 summarizes results about the comparison of final optical densities obtained during stages 1, 2 and 3, for conditions 10, 20 and 30% v/v of PW. As it can be seen, higher final optical densities were obtained after stage 3 compared to stages 1 and 2, suggesting that acclimation had a positive effect on final concentration of microalgae. It can be hypothesized that microalgae become accustomed to these complex culture medium and the potential inhibitory compounds, being less stressed, spending less and less energy to counter it and being able to use the available carbon to multiply more. As shown by Osundeko et al. [41], stresses can induce the accumulation of storage carbon metabolites, such as triacylglycerol and starch, reducing microalgal growth. However, they have shown that acclimated cells to wastewater showed better growth rates and biomass productivities.

3.7. Nitrogen removal in produced water and digestate

Tests revealed a complete removal of ammonium nitrogen during cultures due to consumption by algae and bacteria and stripping (as approximately 5% of nitrogen was in the form of ammonia at pH of 7.9 and 24 °C [42]). Removal kinetics were constant for each condition independently of the growth phases (data not shown). Considering these results, it can be supposed that nitrogen was a limiting nutrient during batch tests, especially for low PW loading (0, 10 and 20% v/v) where nitrogen was removed from the medium before reaching stationary phase. Average removal rates ($rN\text{-}NH_4^+$, $mg \cdot L^{-1} \cdot day^{-1}$) for stage 1 and 2 are shown in Fig. 9.

Concerning stage 1, removal was 5.9 ± 0.2 $mg \cdot L^{-1} \cdot day^{-1}$ for the condition 0% v/v of PW and 3.6 ± 0.4 $mg \cdot L^{-1} \cdot day^{-1}$ for the conditions 10, 20 and 30% v/v of PW. These results confirmed that an increase of PW loading is detrimental to culture growth. Except for the condition with 30% v/v of PW, ammonium assimilation during stage 2 was higher than in stage 1, reaching 5.6 ± 0.2 and 6.3 ± 0.1 $mg \cdot L^{-1} \cdot day^{-1}$ for 10 and 20% v/v of PW, and approximately

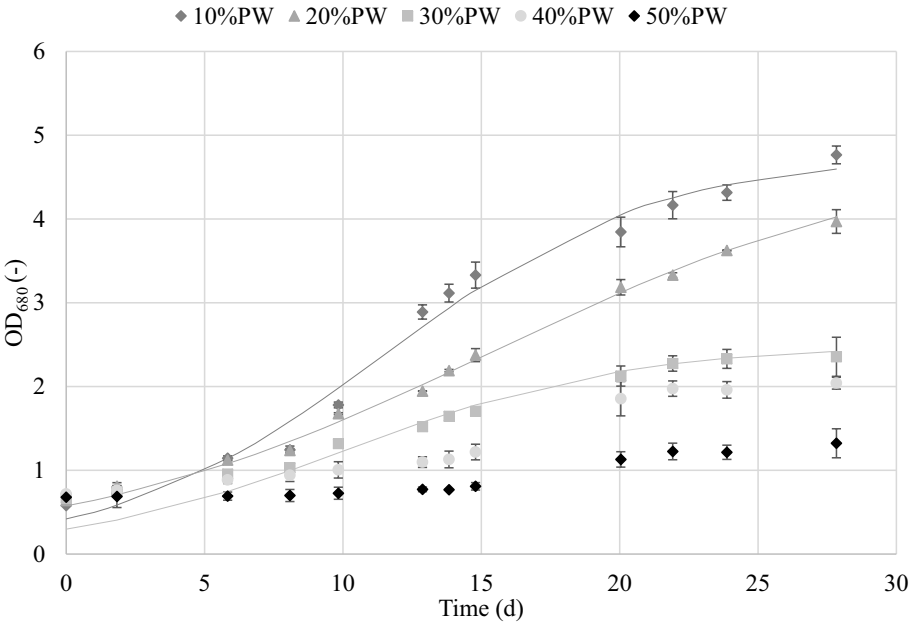

Fig. 6. Evolution of optical density at 680 nm (OD_{680}) during cultivation experiments of stage 2 at different produced water (PW) loadings. Points correspond to experimental data, lines correspond to growth modeling using the modified Logistic function. Values correspond to mean ± standard deviation of measurement performed in duplicate.

Table 4
Conditions and growth parameters of *Nannochloropsis oculata* calculated during stage 2. Values correspond to mean ± standard deviation of measurement performed in duplicate. PW: produced water; OD: optical density.

PW loading (%)	Salinity ($g{\cdot}L^{-1}$)	Maximal OD_{680}	Time until maximal OD (days)	Estimated maximal cell concentration (10^7 cells·mL^{-1})	Growth rate (day^{-1})	Doubling time (days)
10	39.7 ± 0.2	4.77 ± 0.11	28	9.3 ± 0.2	0.24 ± 0.01	2.9 ± 0.2
20	47.7 ± 0.1	3.97 ± 0.14	28	7.9 ± 0.3	0.16 ± 0.00	4.4 ± 0.1
30	56.7 ± 0.1	2.36 ± 0.23	22	4.8 ± 0.2	0.12 ± 0.00	5.6 ± 0.1
40	65.0 ± 0.0	2.04 ± 0.07	22	4.2 ± 0.2	–	–
50	73.9 ± 0.1	1.23 ± 0.10	22	2.8 ± 0.2	–	–

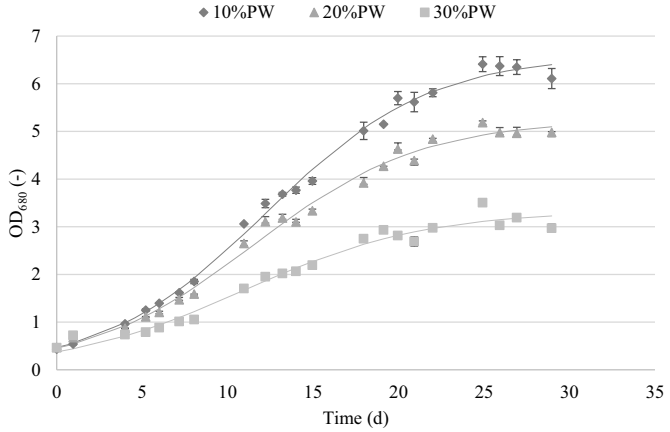

Fig. 7. Evolution of optical density at 680 nm (OD_{680}) during cultivation experiments of stage 3 at different produced water (PW) loadings. Points correspond to experimental data, lines correspond to growth modeling using the modified Logistic function. Values correspond to mean ± standard deviation of measurement performed in duplicate.

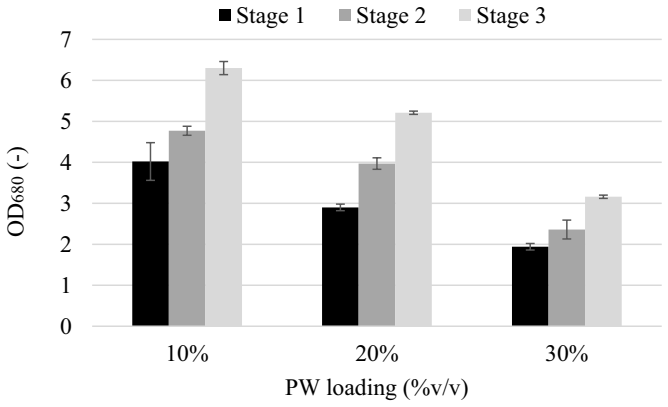

Fig. 8. Final optical densities at 680 nm (OD_{680}) obtained during stages 1, 2 and 3 for 10, 20 and 30%v/v of produced water (PW) loading. Values correspond to mean ± standard deviation of measurement performed in duplicate.

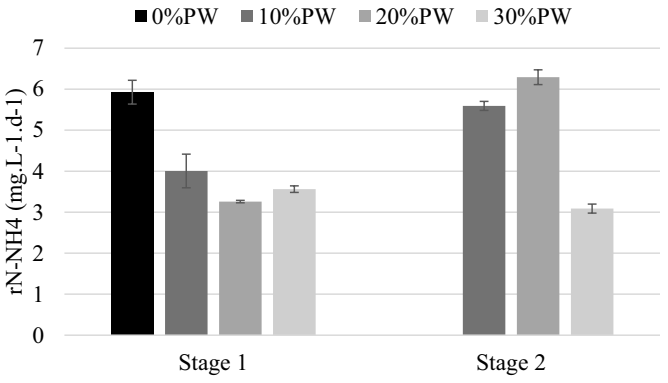

Fig. 9. Average nitrogen removal rates (rN-NH_4^+, $mg{\cdot}L^{-1}{\cdot}day^{-1}$) for stage 1 and 2. Values correspond to mean ± standard deviation of measurement performed in duplicate. PW: produced water.

3.1 $mg{\cdot}L^{-1}{\cdot}day^{-1}$ for 30% v/v of PW. It can be hypothesized that inoculum of stage 2 coming from stage 1, microalgae were acclimated to ammonium as nitrogen source and consume it faster, whereas inoculum for stage 1 being cultivated on F medium using nitrates as nitrogen source. These results are coherent with nitrogen assimilation found in literature for *Nannochloropsis* sp. For example, Cai et al. [40] also reported a nitrogen removal of 100% for digestate loadings of 3% and 6% v/v mixed with marine F/2 medium. Nitrogen removal performances decreased to 87% as the digestate loading increased from 6 to 24% v/v. These results show that *Nannochloropsis* sp. are perfectly able to assimilate nitrogen from PW and liquid digestate and it seemed even more effective after an acclimation step.

3.8. Chemical oxygen demand removal in produced water and digestate

In order to estimate the bioremediation efficiency of PW and liquid digestate by microalgae, soluble COD was monitored during stage 1 and 2. Fig. 10 shows the % of COD removal efficiencies in culture tests monitored at day 2 and at the end of stage 1 and 2.

These results are coherent with those obtained during control tests in absence of microalgae (data not shown), as approximately 30–40% of COD was removed during the first 2 days for media composed of 20% v/v of PW and 5% v/v liquid digestate. This is in part due to a degradation by bacteria brought by liquid digestate and PW [43] and/or a volatilization of BTEX and VFA, which represented < 12% (VFA) and 2% (BTEX) of initial soluble COD. It is noteworthy that for both stage 1 and 2 the % of COD removal during the two first days increased with the increase of the % of PW loading (up to 43%), probably due to an

Table 5
Conditions and growth parameters of *Nannochloropsis oculata* calculated during stage 3. Values correspond to mean ± standard deviation of measurement performed in duplicate. PW: produced water; OD: optical density.

PW loading (%)	Salinity ($g{\cdot}L^{-1}$)	Maximal OD_{680}	Time until maximal OD (days)	Estimated Maximal cell concentration (10^7 cells·mL^{-1})	Growth rate (day^{-1})	Doubling time (days)
10	39.8 ± 0.2	6.41 ± 0.16	25	12.4 ± 0.3	0.35 ± 0.01	2.0 ± 0.1
20	48.3 ± 0.1	5.18 ± 0.04	25	10.0 ± 0.1	0.27 ± 0.00	2.6 ± 0.0
30	57.7 ± 0.1	3.50 ± 0.04	25	7.0 ± 0.1	0.16 ± 0.00	4.4 ± 0.1

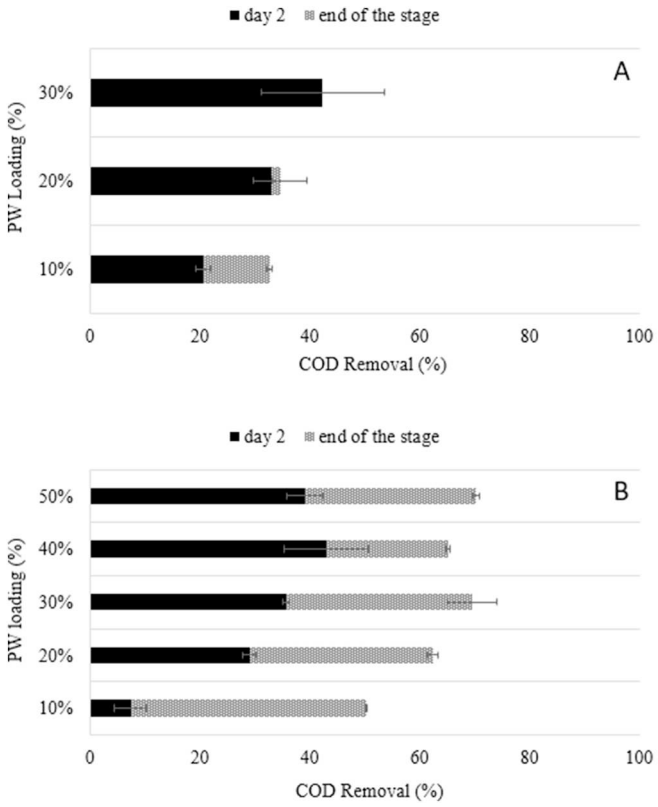

Fig. 10. % of chemical oxygen demand (COD) removal efficiencies during stage 1 (A) and stage 2 (B). Values correspond to mean ± standard deviation of measurement performed in duplicate. PW: produced water.

increase in volatilize compounds in the culture medium and remained constant for both stages. Moreover, the total COD removal was higher during stage 2, compared to stage 1, as 50 to 70% of removal were reached at the end of the stage, resulting in an increase of 20–30% of removal for each test condition, even for 40 and 50% v/v of PW conditions with very high salinity, due to degradation by microalgae and bacteria. Despite the high variability of test conditions, similar results for 50% v/v PW were obtained by Ammar et al. [16] who reported 90, 72 and 54% of COD removal for 10, 25 and 50% v/v PW loading respectively, with a decrease in % of COD removal with the increase of the % of PW loading. These results show that marine microalgae *Nannochloropsis oculata* in combination with bacterial population appeared to be a potential candidate to bioremediate PW and liquid digestate after one or several step of acclimation.

3.9. Assimilation/adsorption of metals in Nannochloropsis oculata

Metal analysis was conducted at the end of stage 1 on both centrifugated microalgae residues and supernatants in order to assess the mass balance. Because of low concentrations of most metals in the initial culture medium, the mass balance was only possible on calcium (Ca), magnesium (Mg) and iron (Fe). Mass balance was satisfactory, as 85–115% of Ca, Mg and Fe were found at the end of stage 1, compared to the initial concentrations. Ca and Mg did not precipitate or accumulate in microalgae residues as they were found in supernatants after centrifugation. On the contrary, Fe has been chemically precipitated and/or assimilated by microalgae. Indeed, initial concentrations of iron in the media were 2.4, 4.8 and 7.2 $mg_{Fe} \cdot L^{-1}$ for conditions 10, 20 and 30% v/v of PW. After growth, 100%, 91% and 95% of iron (for 10, 20 and 30% v/v of PW, respectively) were found in microalgal residues rather than in supernatants at the end of these tests.

4. Conclusions

This study investigated the feasibility of using PW complemented with liquid digestate and seawater as a culture medium for the marine green microalgae *Nannochloropsis oculata*. Soluble COD removal, ammonium nitrogen assimilation rates and metals accumulations were also monitored to determine the potential ability of *N. oculata* to bioremediate PW and liquid digestate. Experiments had been performed by testing media composed of different PW loadings (from 0 to 50% v/v) supplemented with liquid digestate (5% v/v) and seawater. Results allowed us to conclude:

- PW and liquid digestate diluted with seawater are good media for growing microalgae instead of fresh water. However, high salinity ($> 60\ g \cdot L^{-1}$) could limit the growth of *Nannochloropsis oculata*.
- Diluted liquid digestate is a source of ammonium which, if supplemented with PO_4^{3-}, allowed a better microalgae growth than commercial synthetic medium F/2 and permitted to obtain two times higher final biomass concentration (i.e. OD_{680} of 6 and 3 for diluted liquid digestate and F/2 medium, respectively).
- Acclimation of microalgae had a positive effect on microalgae productivity and their growth kinetics.
- Mixed culture of *N. oculata* and bacteria appeared to be a potential candidate to bioremediate PW and liquid digestate: soluble COD removal up to 70% was observed after acclimation, in part (30–40%) during the first two days of the culture, due to a degradation of bacteria and due to the volatilization of some compound as VFA (11%) and/or BTEX (2%) in digestate and PW respectively. Ammonium was assimilated and/or stripped up to 100% with higher kinetics at low PW loading (10 and 20% v/v). Finally, an iron assimilation and/or precipitation up to 100% was observed during stage 1.

Further studies should be conducted to integrate solubilized solid digestate as a complement of phosphorus in the culture medium and to deep understand the COD removal mechanisms observed during the first days of culture.

CRediT authorship contribution statement

Aurélien Parsy: Conceptualization, Investigation. **Cecilia Sambusiti:** Supervision. **Patrick Baldoni-Andrey:** Project administration. **Thomas Elan:** Funding acquisition, Writing - review & editing, Supervision. **Frédéric Périé:** Funding acquisition, Writing - review & editing, Supervision.

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.

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Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this study.

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