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Comparative evaluation of microalgae strains for CO_2 capture purposes

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ABSTRACT

In this paper we have compared eleven microalgae/cyanobacteria strains used for the development of a CO_2 capture process. Firstly, we studied the tolerance of the selected strains to the water quality available at the production site. The results confirmed that no toxins were present in the water used; in addition, we confirmed that fertilizers could be utilised as the nutrient source instead of pure chemicals. Secondly, the strains were evaluated in terms of growth rate, biomass productivity and photosynthetic efficiency concluding that *Scenedesmus almeriensis*, *Neochloris oleoabundans* and bloom from the River Seine were the most productive, above 1.0 $g{\cdot}L^{-1}{\cdot}day^{-1}$. Thirdly, we determined the biochemical composition of the biomass with the results showing that most of the strains mainly accumulate carbohydrates in the stationary phase, over 60% d.wt.; the exceptions were *Neochloris oleoabundans* and *Chlorella vulgaris,* which accumulate lipids, above 20% d.wt. In any case, the performance of the microalgae strains was better than that of cyanobacteria both in terms of biomass productivity and the biochemical composition; consequently, using these types of microorganisms is recommended. By considering a fixed value for the main biomass components, we concluded that the most promising strains were *Scenedesmus almeriensis*, *Neochloris oleoabundans* and bloom from the River Seine, yielding a biomass value above 0.6 $€{\cdot}kg^{-1}$ and an economic value higher than 0.7 $€{\cdot}m^{-3}{\cdot}day^{-1}$. These figures confirm that, in order to obtain profitable CO_2 capture processes and to develop more efficient production systems that reduce current production costs, coupling with wastewater treatment schemes is required.

1. Introduction

The release of greenhouse gases, especially CO_2, is a well-recognized problem affecting the climate worldwide. To mitigate this problem, different strategies have been proposed and recommended for implementation, from reductions in fossil fuel use to improved efficiency in current energy conversion systems, as well as the capture and recycling/storage of the CO_2 released [1] With regard to CO_2 capture, different technologies have been put forward, most based on absorption-desorption processes using amines; whilst others use solid adsorbents to separate the CO_2 from the flue gases prior to storage. These systems consume large amounts of energy, they also face corrosion and operation problems related to the different contaminants contained in the flue gases [2]. In fact, biofixation of CO_2 by microalgae is being extensively investigated as part of the greenhouse gas reduction strategy [3–5]. Carbon dioxide from the atmosphere or from flue gases can be used to boost microalgae growth. Several studies have shown that microalgae have better CO_2 fixation ability (10–50 times more) than terrestrial plants [6,7].

As an alternative, the utilization of biological systems requires less energy but capacity is lower. For this application, microalgae are recommended because of their high productivity as well as other advantages [8,9]. Multiple studies have been performed with this objective in mind, all concluding that the relevant strains to be used, the nutrient supply and the overall cost of the technology are bottlenecks for the commercial development of microalgae-based CO_2 capture processes [3,10–12].

Due to their unique properties, microalgae (including cyanobacteria) represent an extremely diverse group of organisms, considered as promising feedstocks for applications in food and feed production, bioactive pharmaceuticals, nutraceuticals, functional foods, and even wastewater treatment, CO_2 capture and biofuel production [13]. Microalgae are some of the oldest living microorganisms on Earth [14]. They grow at an exceptionally fast rate, up to 100 times faster than terrestrial plants, and they can double their biomass in less than a day, thus making productivities up to 100 tn/ha·year possible [15]. To do so, microalgae cultures must be provided with large amounts of nutrients - for each tonne of biomass produced, it takes up to 2 tn CO_2,

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100 kg N and 10 kg P. Large volumes of water are also required, more than 1 m^3 of reactor volume per kg of microalgae biomass in addition to adequate environmental conditions (light, temperature, etc.). The basic environmental factors such as pH, temperature and light intensity have a great influence on the growth dynamics of microalgae as do the nutrients [16]. Initially, lab-scale cultivation has to be carried out to maximize cell growth by setting up the natural conditions inside the laboratory [17]. The aim of this work was to establish these initial conditions for the development of a microalgae-based CO_2 capture process. The first step was to analyse the boundary conditions, such as water quality, at the location where the flue gases are produced. With this information, a selection of suitable microalgae strains was chosen for evaluation under laboratory conditions simulating the targeted location. The biochemical composition of the produced biomass was determined in addition to the photosynthetic efficiency. Finally, we selected the most suitable strains based on their productivity under the conditions/water quality prevailing at the selected location; this was combined with the biochemical composition of the produced biomass and a brief economic analysis to support decision makers.

2. Materials and methods

2.1. Location and characteristics of the flue gases

The facility releasing the flue gases is located in Paris, close to the River Seine. It is a wash incinerator treating the city's organic residues to produce heat and electricity. The temperature in the oven is up to 1000 °C to minimise the release of organic pollutants. The flue gases are pre-treated to remove particles, nitrogen oxides and other contaminants prior to release into the environment. No CO_2 removal processes are implemented, which is why this research was carried out. The site utilises large amounts of the water available from the River Seine for cooling purposes. The river's water composition was unknown so an initial characterisation was undertaken (Table 1). The organic load of the water was low, COD being lower than 100 mg/L. Based on its chemical composition, an artificial version of the river water was prepared to perform the experiments although the potential toxicity/relevance of non-analysed compounds was evaluated using natural water taken from the River Seine.

2.2. Microorganisms and culture media

Different microorganisms, microalgae and cyanobacteria were preselected according to previous experience and the bibliography [18–20]. We selected only robust strains suitable for large-scale production under non-optimally controlled conditions (Table 2). Some of the microorganisms were already available at the University of Almeria whilst others were obtained from official culture collections, mainly from the Culture Collection of Algae and Protozoa (Oban, Scotland). Inoculum from all the strains were kept under controlled conditions in 1 L flasks, at 20 °C, under constant illumination at 200 $\mu E \cdot m^{-2} \cdot s^{-1}$ provided by fluorescent lamps, with constant aeration at 0.1 v/v/min with no CO_2 supply in a standard Arnon culture medium. The standard culture medium was prepared using nutrients of analytical grade and distilled water, which was autoclaved at 121 °C for 15 min. The inoculum cultures were monitored by microscopic observation using a Leica CME microscope 40X/0.65 to verify non-contamination.

Table 1
Chemical composition of natural water from the River Seine and the artificial Seine river water prepared to perform some of the experiments.

Components, mg/L	River Seine - Natural	River Seine - Artificial
HCO_3^-	256.27	256.27
Cl^-	99.76	99.76
Na^+	30.00	96.63
K^+	10.90	10.90
Ca^{2+}	104.00	41.72
Mg^{2+}	5.40	5.40
PO_4^{-3}	1.09	0.90
NH_4^+	1.05	1.05
Fe^{3+}	0.40	0.40
SO_4^{-2}	0.00	0.34

Table 2
List of microorganisms selected, including microalgae and cyanobacteria, following the bibliographic revision.

Species	Microalgae/cyanobacterium	Origin
River Seine bloom	Microalgae	River Seine
Scenedesmus almeriensis	Microalga	UAL collection
Neochloris oleoabundans	Microalga	UAL collection
Anabaena sp.	Cyanobacterium	CCAP 1403/13
Spirulina platensis	Cyanobacterium	UAL collection
Nostoc commune	Cyanobacterium	CCAP 1453/33
Calothrix scytonemicola	Cyanobacterium	CCAP 1410/12
Scenedesmus dimorphis	Microalga	UAL collection
Chlorella vulgaris	Microalga	CCAP 211/11D
Monoraphidium griffithii	Microalga	CCAP 202/11D
Synechococcus sp.	Cyanobacteria	CCAP 1479/9

2.3. Culture media

Although different culture media are usually proposed for each strain, the main components of most of the culture media reported are quite similar. In this work the composition of the Arnon medium was selected as standard although it was prepared in different ways: (1) Control cultures were developed with Arnon culture medium prepared using pure grade chemicals and distilled water. (2) River Seine natural cultures were developed also with Arnon culture medium prepared using pure grade chemicals but with natural water from the river instead of distilled water, thus allowing us to identify the adverse effects of any toxic substances present in the natural Seine river water. (3) To validate the composition of the artificial Seine river water, we performed experiments also using Arnon culture prepared with pure grade chemicals but using artificial river water. (4) Finally, to evaluate the possibility of reducing the culture medium cost for large-scale use, the culture medium was prepared using artificial water from the River Seine plus commercial fertilizers providing the equivalent nutrients as supplied by the Arnon medium. The composition of the Arnon and fertilizer media are shown in Table 3.

2.4. Photobioreactors and operation mode

Experiments were performed in bubble-column photobioreactors (~ 300 mL) aerated at 0.2 v/v/min with pH controlled at 8.0 by on-

Table 3
Chemical composition of the Arnon and fertilizer media used.

Pure salts, mg/L	Arnon medium	Fertilizer, mg/L	Fertilizer medium
$NaNO_3$	850.00	$Ca(NO_3)_2$	640.00
K_2HPO_4	696.00	$NaNO_3$	260.00
$FeSO_4 \cdot 7H_2O$	24.91	$MgSO_4$	180.00
KOH	19.05	KH_2PO_4	140.00
NaCl	117.00	Fe (Fe chelate)	0.66
$CaCl_2 \cdot 2H_2O$	15.00	Welgro, ml/L	44.00
$MgSO_4 \cdot 7H_2O$	124.00		
$CoCl_2 \cdot 6H_2O$	0.04		
$CuSO_4 \cdot 5H_2O$	0.08		
$ZnSO_4 \cdot 7H_2O$	0.22		
$MnCl_2 4H_2O$	1.81		
H_3BO_4	2.86		
$Na_2MoO_4 \cdot 2H_2O$	1.26		
$NaVO_3$	0.24		

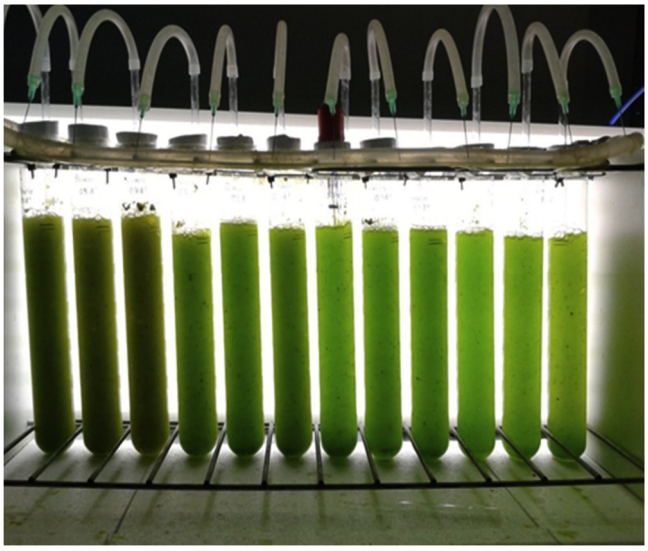

Fig. 1. Image of the bubble-column photobioreactors used during the experiments.

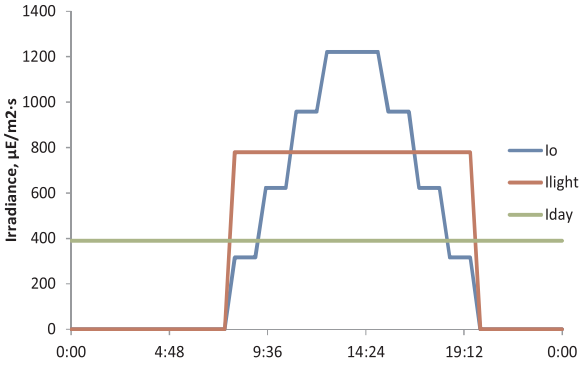

Fig. 2. Daily variation in irradiance on the reactor surface during the experiments.

demand injection of pure CO_2 in the airstream entering the reactors. The temperature inside the reactors was kept at 25 °C by controlling the temperature of the chamber in which the reactors are located (Fig. 1). A total of 15 bubble-column reactors were used and each experiment was tested in triplicate. The reactors are illuminated artificially using fluorescent lamps that are automatically turned on or off to simulate the circadian solar cycle (Fig. 2). Irradiance on the reactors surface (Io) varied throughout the day from zero to 1200 $\mu E \cdot m^{-2} \cdot s^{-1}$ at noon - using these values, a mean irradiance for the light period (I_{light}) of 780 $\mu E \cdot m^{-2} \cdot s^{-1}$ was obtained. On a 24 h basis, the mean irradiance on the reactor surface (I_{day}) was 390 $\mu E \cdot m^{-2} \cdot s^{-1}$.

Experiments were performed in batch mode. For this, we used inoculum from previous cultures developed in flasks using the same culture medium but without pH control and under continuous illumination at 200 $\mu E \cdot m^{-2} \cdot s^{-1}$. The volume of inoculum supplied to the reactors at the beginning of the experiment was 10% of the total culture volume in the bubble column; this was done to achieve a low biomass concentration from the start so as to correctly evaluate the growth curve. Once the reactor was inoculated, it was monitored daily measuring the biomass concentration and the fluorescence of chlorophylls. Water evaporation was compensated for each day with distilled water to avoid changes in conductivity or of any nutrient in the culture broth. At the end of the experiment, when the biomass concentration reached a maximal value, the entire culture was harvested and the biomass was stored for further biochemical composition analysis.

2.5. Analytical methods

The cultures were examined daily under a microscope, an Olympus CH20 (Olympus Corp., USA), to evaluate the cell status and to detect possible contamination. Images of the cultures were photographed for further use. Absorbance and turbidity were measured daily to monitor the evolution of the cultures. The dry weight biomass concentration (Cb) was measured by filtering 100 ml of culture through 1 µm filters and drying it at 80 °C in an oven over a 24 h period; this measurement was performed at the end of the culture. The dry weight biomass concentration values during the batch experiments were calculated from absorbance/turbidity measurements using the correlation obtained at the end of the batch culture. Biomass productivity was calculated as the product of the biomass concentration; this was done multiplied by the growth rate each day. The cell status was checked daily by measuring the chlorophyll fluorescence (Fv/Fm) ratio with a fluorometer (AquaPen AP 100, Photon System Instruments, The Czech Republic). For this, the cells were adapted to the dark for 15 min prior to measurement. Absorbance in the visible range (400–700 nm) was measured daily using a double-beam Helios Alpha spectrophotometer and the extinction coefficient (Ka) was calculated by dividing the average absorbance value by the biomass concentration (Cb) and the cuvette's light path (p) (Eq. (1)):

$$K_a = \frac{Abs}{C_b \cdot p} \tag{1}$$

The average irradiance inside the culture (Iav) was calculated as a function of the irradiance at the surface (Io), the biomass extinction coefficient (Ka), the biomass concentration (Cb) and the light path inside the reactor (p) (Eq. (2)) [21]. Because mean daily values were considered, irradiance during the light period (I_{light}) was used as the irradiance on the reactor surface to calculate the mean daily irradiance.

$$I_{av} = \frac{I_{light}}{K_a \cdot C_b \cdot P} \cdot (1 - \exp(-K_a \cdot C_b \cdot p)) \tag{2}$$

Quantum yield (ΨE) is defined as the amount of biomass generated by a unit of radiation (usually a mole of photons) absorbed by the culture. Since this represents the ratio of biomass generation to absorbed photon flux, it can be calculated using Eq. (3) [22]. The photon flux absorbed through the reactor volume (F_{vol}) is calculated from the average irradiance on a culture volume basis using Eq. (4) [22].

$$\Psi_E = \frac{P_b}{F_{vol}} \tag{3}$$

$$F_{vol} = I_{av} \cdot K_a \cdot C_b \tag{4}$$

Photosynthetic efficiency (PE) is the fraction of energy fixed into biomass as a function of the combustion heat of the biomass that was considered constant (Qb = 20 MJ/kg) (Eq. (5)) [22].

$$PE = \frac{P_b \cdot Q_b}{F_{vol}} \tag{5}$$

With regards to biochemical composition, freeze-dried biomass taken at the end of the batch culture was analysed. Lipids were determined gravimetrically from an extract obtained with chloroform:methanol (2:1) (v/v) [23]. The protein content was determined using the modified Lowry method [24]. The moisture content was determined by weight losses after 24 h at 80 °C, whereas the ash content was determined by calcination at 550 °C for 6 h. The carbohydrate content of the biomass was determined as the difference remaining from 100% after taking away the protein, lipid and ash content.

3. Results and discussion

Biological CO_2 capture processes have been studied as an alternative way to reduce global warming [25]. Given that microalgae biomass

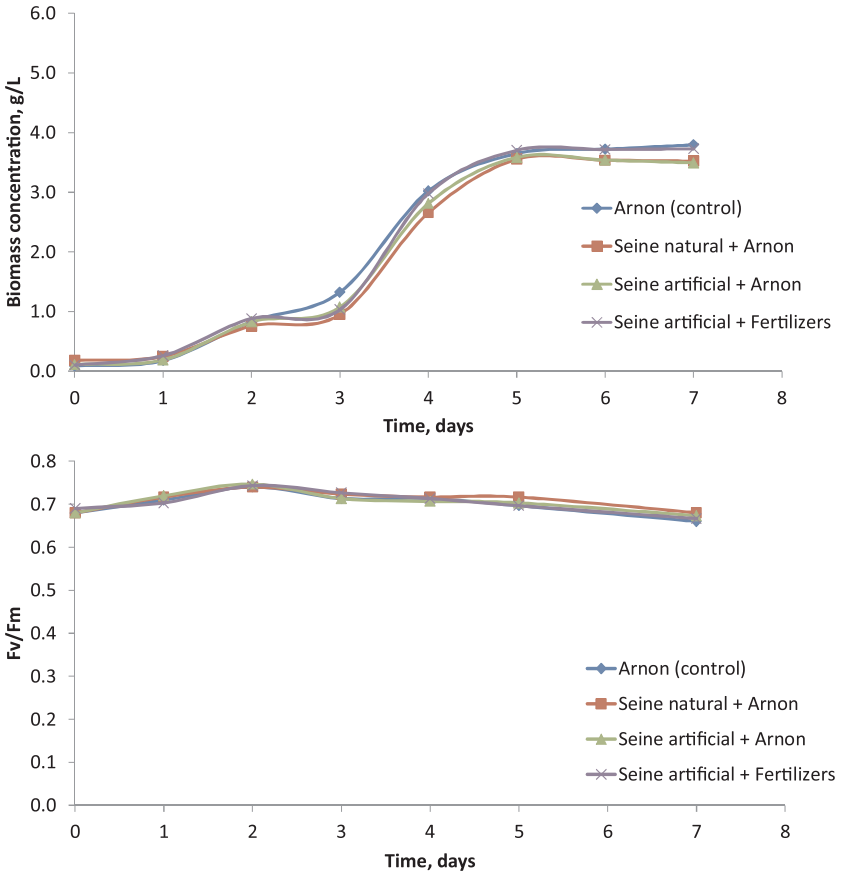

Fig. 3. Experimental data obtained during batch culture of the bloom from the River Seine in the different culture media assayed.

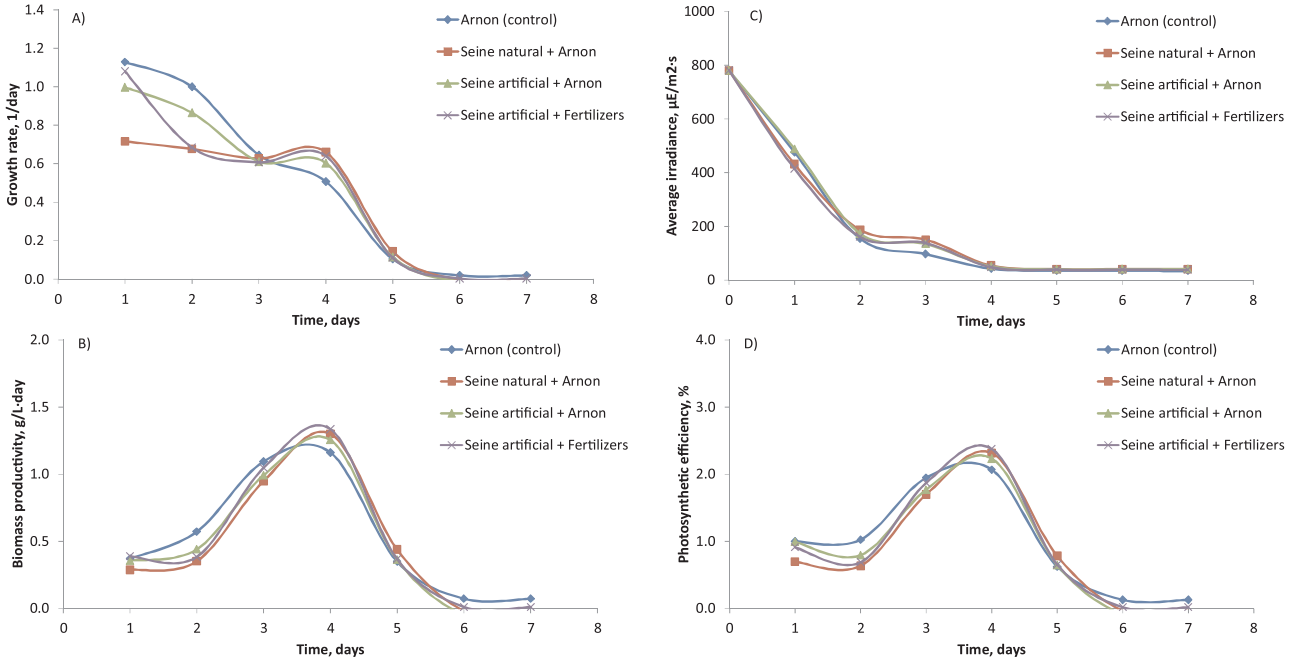

Fig. 4. Calculated values of the growth rate, biomass productivity, average irradiance and photosynthetic efficiency from experimental measurements obtained during batch culture of the bloom from the River Seine in the different culture media evaluated.

contains approximately 50% carbon, then up to 1.83 kg of CO_2 can be captured per kg of biomass produced. To maximise the CO_2 fixation capacity, one must select highly productive and robust strains because the amount of CO_2 fixed is directly proportional to the amount of biomass produced. Additionally, the biochemical composition of the biomass must be as valuable as possible although this is a second-tier variable that mainly determines the subsequent uses of the biomass produced. To achieve a suitable commercial process for CO_2 capture, it is necessary to produce valuable biomass otherwise the production cost will be higher than the CO_2 emission taxes [10].

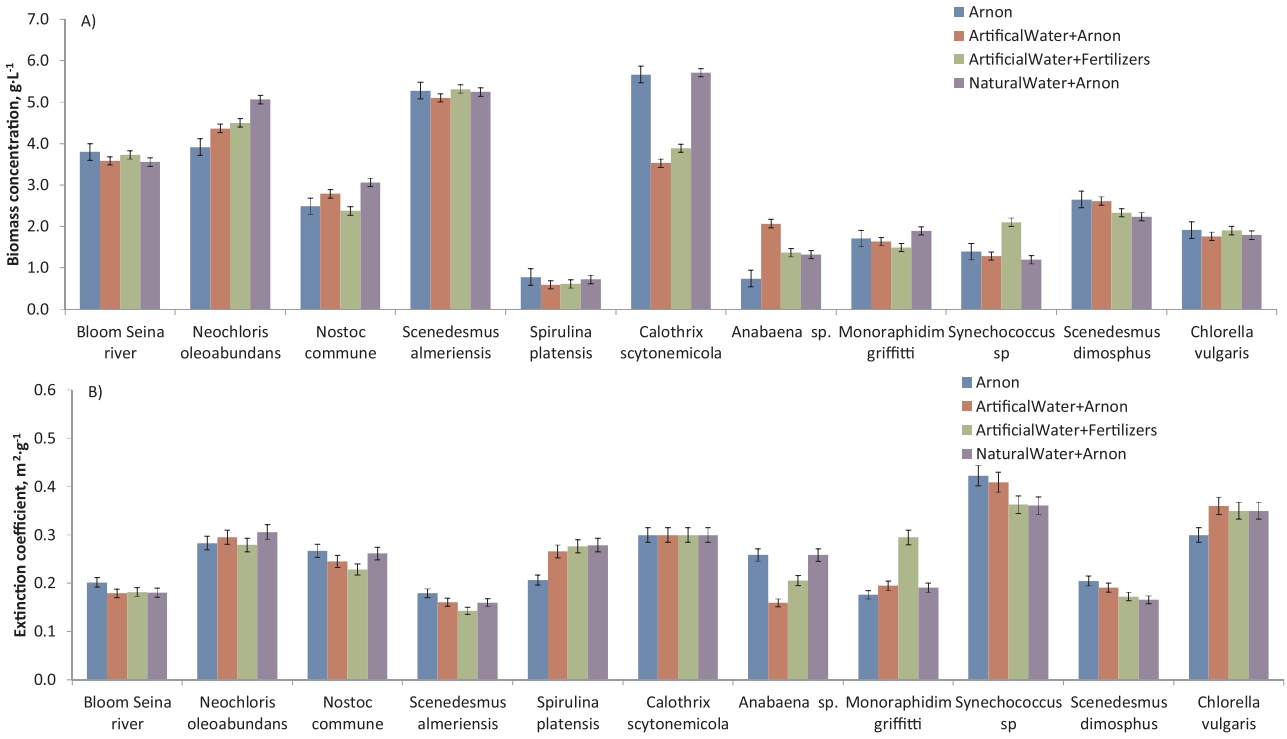

Fig. 5. Experimental biomass concentration and extinction coefficient values of the biomass at stationary phase for the evaluated strains in the different culture media assayed.

Concerning strain robustness, the primary requirement is that it can be grown in the water available at the facility's location - in this case, the River Seine. Then, suitable culture media need to be defined based on the available water. To do this, we determined the chemical composition of the Seine river water (Table 1). The results confirmed that this freshwater contains only small amounts of the main nutrients required to produce microalgae, such as C, N and P, but high concentrations of other minor nutrients. Based on this composition and considering the reported coefficient yield values for carbon (0.5 gC/$g_{biomass}$), nitrogen (0.05 g N/$g_{biomass}$), and phosphorus (0.01 gC/$g_{biomass}$) [26], it was evident that additional nutrients were required to use the water from the River Seine. Indeed, only 16 and 109 mg of biomass would be produced based on the river's N and P content. In terms of the river's carbon content, up to only 100 mg of biomass would be produced although this bottleneck could be overcome by on-demand CO_2 injection thus improving on the real culture conditions. The content of the other minor components, such as Ca^{2+}, Mg^{2+}, Fe^{3+} etc., was high enough based on the other culture media proposed in the literature [27]. To solve the nutrient limitation problem, mainly for N and P, Arnon culture medium was selected; this was prepared using pure chemicals or regular fertilizers (Table 3). Based on the N and P content of Arnon medium, and using the same coefficient yields as previously indicated, one can calculate that up to 2.8 and 12.7 $g{\cdot}L^{-1}$ of biomass can be produced. These results confirm that Arnon medium contains too much phosphorus; hence, when preparing the culture medium using fertilizers, the phosphorus content was reduced. In this way, when using the fertilizer medium, up to 2.2 and 3.2 $g{\cdot}L^{-1}$ of biomass can be produced based on the N and P content.

Once we defined the culture media and the water quality, the experiments were performed; thus batch cultures of the selected stains were developed in the defined culture media. The data from experiments using bloom from the River Seine are shown as an example (Fig. 3). The data show that, during the batch culture, the biomass concentration increased to 3.7 $g{\cdot}L^{-1}$; no differences were observed in relation to the nutrient source or water quality used (Fig. 3A). Furthermore, we observed no variation in the fluorescence of chlorophylls, Fv/Fm, over time or due to the different culture media or water quality used (Fig. 3B). Microscopic observations show that the prevailing strain in the bloom culture was *Scenedesmus* sp. These results confirm that water from the River Seine is adequate for growing this naturally selected strain and that the water from the river is not toxic to it. Moreover, the growth rate was similar using Arnon medium prepared with pure chemicals or medium prepared using fertilizers as the nutrient source. From these figures, the additional relevant parameters can be calculated (Fig. 4). One can observe that the growth rate was maximal at the beginning of the cultures when the average irradiance was also maximal due to the low biomass concentration inside the culture; however, this reduced over time to zero in stationary phase when the light availability reached minimum irradiance for maintenance (Fig. 4A). Due to the opposite variation in growth rate and biomass concentration, the biomass productivity shows a Gaussian-type curve, achieving a maximal value corresponding to the expected maximal biomass productivity in continuous mode (Fig. 4B). Moreover, the optimal conditions for achieving this maximal biomass productivity can be estimated from the figures, corresponding to the biomass concentration, growth rate and average irradiance taking place at the time the maximal biomass productivity was obtained (Fig. 4C). In any case, Fig. 4 shows that for bloom from the River Seine, the different culture media tested did not have a large or relevant effect on these parameters. Thus, a maximal growth rate of 1.1 day^{-1} was obtained at an average irradiance of 800 $\mu E{\cdot}m^{-2}{\cdot}s^{-1}$, whereas the maximal productivity of 1.34 $g{\cdot}L^{-1}{\cdot}day^{-1}$ was achieved at a biomass concentration of 2.9 $g{\cdot}L^{-1}$, corresponding to a growth rate of 0.6 day^{-1} and average irradiance of 48 $\mu E{\cdot}m^{-2}{\cdot}s^{-1}$. These values indicate that the strain would be highly productive, with the optimal dilution rate for continuous operation being 0.6 day^{-1}; although, this can be modified depending on the final photobioreactors and culture conditions used at the large scale. Concerning photosynthetic efficiency, the results show that a maximal value of 2.4% was obtained at maximal productivity, mainly because the illumination was not modified and, therefore, the photosynthetic efficiency was a direct function of biomass productivity (Fig. 4C). In any case, these results confirm that the optimal conditions for biomass

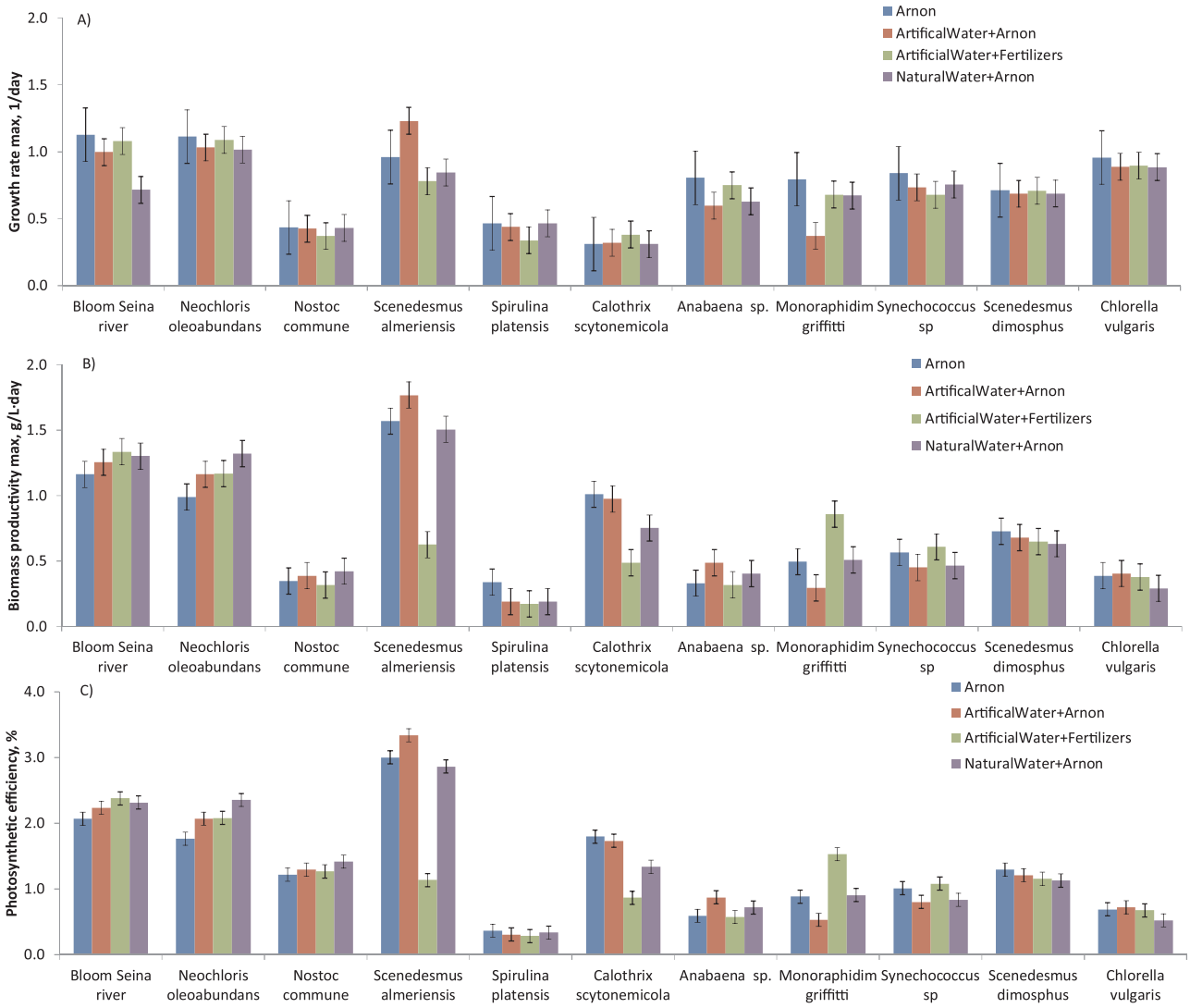

Fig. 6. Calculated values of the maximal growth rate, biomass productivity and photosynthetic efficiency for the evaluated strains in the different culture media assayed.

productivity are also optimal for light utilization efficiency, and because the CO_2 fixation is also a direct function of biomass productivity, they are also optimal for the CO_2 fixation capacity.

When performing the same protocol for all the selected strains, we obtain a valuable comparison of these strain performance under similar culture conditions (Figs. 5 and 6). The data show that the biomass concentration in the stationary phase was much higher for *Scenedesmus almeriensis*, bloom from the River Seine, *Neochloris oleoabundans* and *Calothrix scytonemicola* (above 3.5 $g{\cdot}L^{-1}$) than for the other strains, thus confirming the better performance of these strains under the imposed culture conditions (Fig. 5A). The culture medium was not observed as having a large effect, except in the case of *Calothrix scytonemicola*, which performed better using Arnon medium, both in distilled water and in natural water from the River Seine, than when using other culture media. In any case, we observed no adverse effect using natural Seine river water, whichever the strain, indicating that no toxic substances were present in this water. Moreover, similar results were obtained using fertilizers as when using pure chemicals, indicating that the nutrients provided by the fertilizers allow one to maximise the performance of the selected strains. Biomass concentration values up to 5.6 $g{\cdot}L^{-1}$ were measured for the strains that grew most, which included a higher than expected maximal biomass concentration based on the nutrient content of the culture media tested; this indicates therefore that, at the stationary phase, the coefficient yields might be different to those obtained in the bibliography. Concerning the biomass extinction coefficient, the results indicate that this variable is more related to the strain than to the culture medium used (Fig. 5B). The lowest extinction coefficient values for the biomass, ranging from 0.14 to 0.20 $m^2\,g^{-1}$, were determined for some of the strains that grew most, such as *Scenedesmus almeriensis* and bloom from the River Seine, whereas the highest values, ranging from 0.30 to 0.42 $m^2\,g^{-1}$, were measured for the strains that grew least, such as *Chlorella vulgaris* and *Synechococcus* sp. Although a direct correlation cannot be established between the extinction coefficient and biomass production, a general tendency for this was observed - the higher the extinction coefficient of the biomass, the lower the light penetration inside the cultures - thus with the average irradiance for the same biomass concentration, a lower production capacity would be expected.

To verify the better performance of *Scenedesmus almeriensis*, bloom from the River Seine and *Neochloris oleoabundans,* we analysed the data for the specific growth rate, biomass productivity and photosynthetic efficiency (Fig. 6). The data show that the maximal growth rate was higher than 1.0 day^{-1} only for bloom from the River Seine, *Neochloris oleoabundans*, *Scenedesmus almeriensis* and *Chlorella vulgaris*, whereas it was very low, below 0.5 day^{-1}, for some of the cyanobacteria such as *Nostoc commune*, *Spirulina platensis* and *Calothrix scytonemicola*

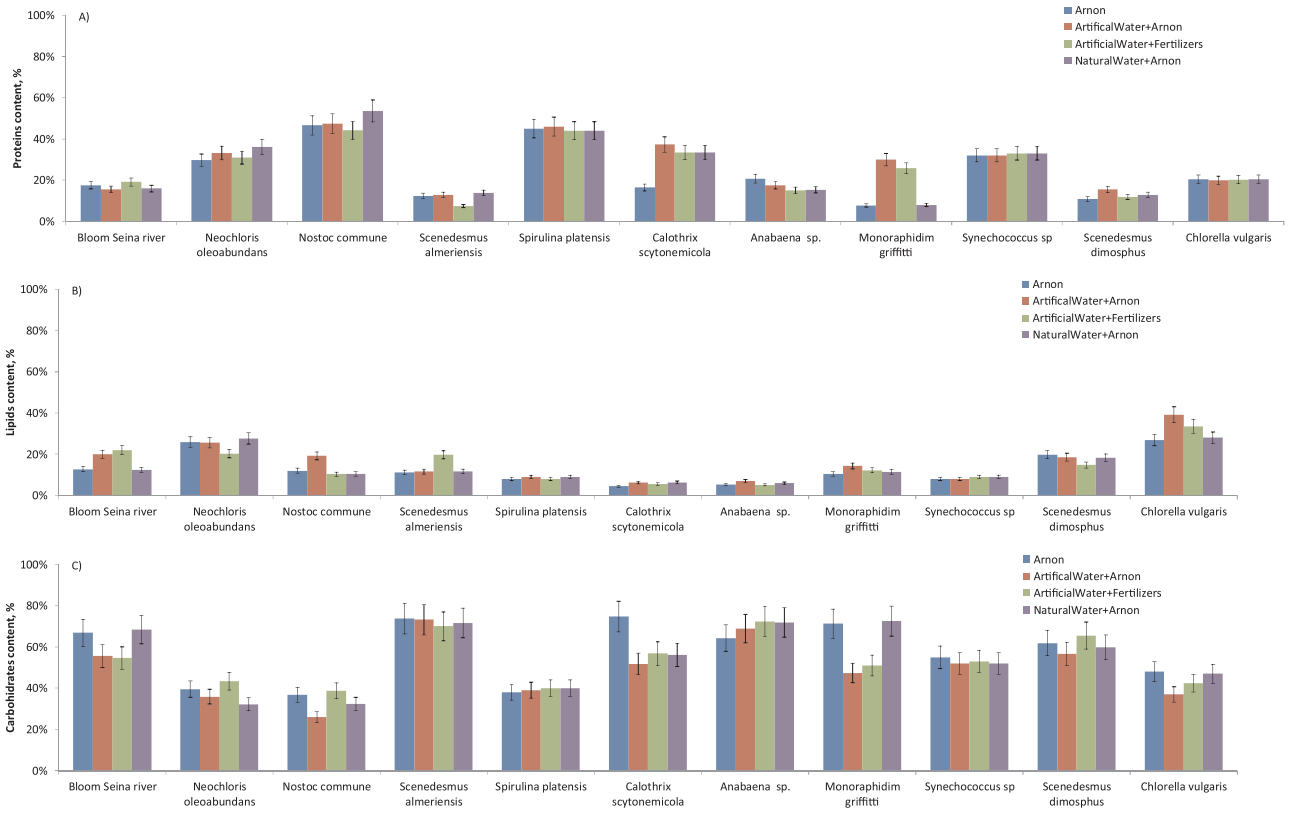

Fig. 7. Biochemical composition of the evaluated strains in the different culture media assayed.

(Fig. 6A). It is important to note that, in the case of *Spirulina platensis,* a high bicarbonate concentration was not supplied, as is usually recommended for this strain; thus it is possible that the selected culture media was not optimal for it. The same phenomena might have occurred for the other slow growing cyanobacteria; however, the culture media were defined as the selection criteria in terms of it being opposite. Regarding the nutrient source and water quality, in general, no adverse effects were observed for these variables regardless of the strain, thus confirming that water from the River Seine contained no substances that were toxic for any of the strains evaluated, and that the culture medium prepared using fertilizers provided the same nutrients as using pure chemicals. Concerning biomass productivity, the most productive strains were *Scenedesmus almeriensis*, bloom from the River Seine and *Neochloris oleoabundans*, which achieved values above 1.0 $g{\cdot}L^{-1}{\cdot}day^{-1}$; in contrast, the biomass productivity of *Chlorella vulgaris* notably reduced (Fig. 6B). Another notable point was the high productivity of *Calothrix scytonemicola* (close to 1.0 $g{\cdot}L^{-1}$) despite its low specific growth rate, indicating that this strain is able to achieve high biomass concentrations under optimal conditions. As before, no significant differences were observed with regard to the nutrient source or the water quality used. Finally, the photosynthetic efficiency results again show that the most productive strains were also the most efficient in terms of light energy conversion, thus *Scenedesmus almeriensis*, bloom from the River Seine and *Neochloris oleoabundans* were the most efficient strains with photosynthetic efficiency values above 2.0% (Fig. 6C). Conversely, *Spirulina platensis*, *Chlorella vulgaris* and *Anabaena* sp. were the least efficient strains under the conditions tested, with values below 0.5%.

The data confirm that *Scenedesmus almeriensis* is a fast growing strain, achieving high biomass concentrations, and biomass productivity equivalent to 2.8 $g{\cdot}L^{-1}{\cdot}day^{-1}$ of CO_2 consumed. This strain is widely reported under outdoor production conditions, including in CO_2 capture processes, as it has demonstrated itself to be robust and suitable for outdoor production, even in non-optimal raceway reactors or using wastewaters as the nutrient source [28,29]. Regarding the bloom from the River Seine, utilising these naturally occurring strains is an interesting strategy to manage robust strains adapted to the environment and they are the most resistant to contaminants and toxic compounds 30,31]. Inoculum was obtained by taking natural Seine river water, providing it with Arnon medium and constant light/aeration until a green colour appeared. Microscopy observation of the inoculum showed that the prevailing strain was *Scenedesmus sp*. The results show behaviour analogous to that observed in *Scenedesmus almeriensis*. The maximal biomass concentration was 3.7 g/L, slightly lower than previously measured for *Scenedesmus almeriensis,* which indicates that the cells prevailing in the bloom culture are slightly less productive than the *Scenedesmus almeriensis* strain from the culture collection. Nevertheless, the performance of the culture was adequate and it would do better in the real River Seine environment to which it is naturally adapted than the *Scenedesmus almeriensis* from the culture collection. In terms of biomass productivity, a maximal value of 1.3 $g{\cdot}L^{-1}{\cdot}day^{-1}$, equivalent to 2.6 $g{\cdot}L^{-1}{\cdot}day^{-1}$ of CO_2 capture capacity, was measured at a biomass concentration of 0.6 $g{\cdot}L^{-1}$, and an average irradiance of 160 $\mu E{\cdot}m^{-2}{\cdot}s^{-1}$; thus confirming its high productivity and light utilization capacity. Finally, *Neochloris oleoabundans* is a small microalga that accumulates large amounts of lipids, up to 50% under non-growing conditions [32]. This strain has been widely reported as a potential biofuel source as it is even able to grow in wastewaters [33,34]. The biomass concentration increases up to values of 5.0 $g{\cdot}L^{-1}$, without any adverse effects when using either natural or artificial Seine river water. With regard to its productivity, the maximal value was 1.32 $g{\cdot}L^{-1}{\cdot}day^{-1}$, equivalent to 2.64 $g{\cdot}L^{-1}{\cdot}day^{-1}$ of CO_2 capture capacity, higher than the reported value of 0.55 $g{\cdot}L^{-1}{\cdot}day^{-1}$ and 0.6 day^{-1} of maximal growth rate using modified BBM [32]. The data confirm that this strain is also a potential candidate for use in CO_2 capture schemes. All in all, these results confirm the higher productivity of microalgae versus cyanobacteria strains, at least under the culture conditions tested. The data also confirm that water from the Seine river, both natural and artificial,

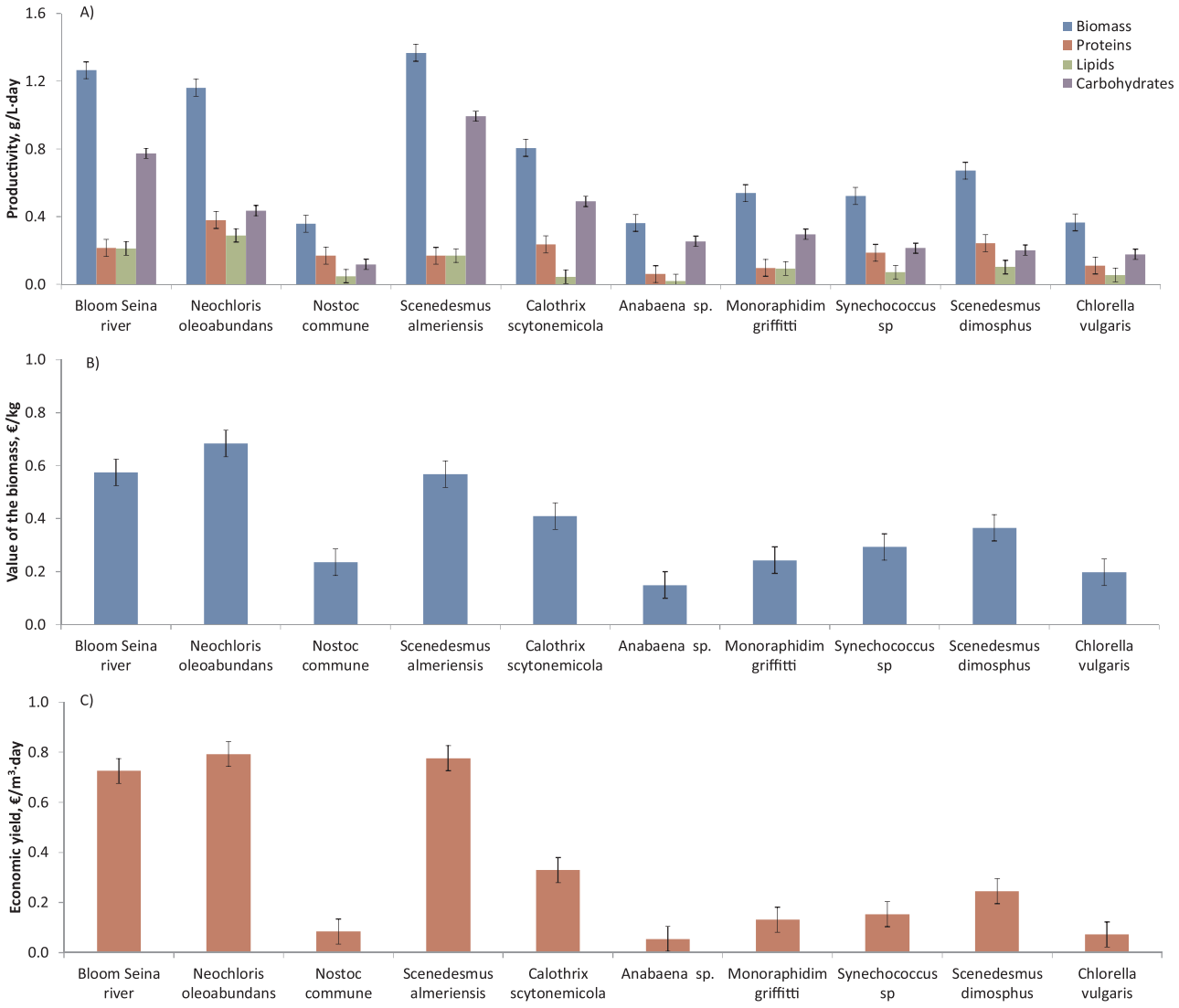

Fig. 8. Specific product productivity, estimated economic value of the biomass and economic yield of the different strains assayed. Values were obtained considering a fixed price for proteins, lipids and carbohydrates and considering the biomass productivity and biochemical composition of the biomass produced under the culture conditions imposed.

are the same and do not contain any substances that are toxic to the production of the selected strains. Additionally, it was confirmed that the culture medium prepared using fertilizers is equivalent but cheaper than that prepared using pure chemicals. Regarding the photosynthetic efficiency, the results confirm that, in microalgae cells, it ranges from 2 to 3% whereas in cyanobacteria, it ranges from 0.5 to 1.5%. Based on this parameter, it is more efficient to produce microalgae than cyanobacteria, and this efficiency can be even further increased by optimizing the culture conditions and reactor design for the final selected strain.

In addition to the biomass production capacity, the biochemical composition of the biomass must also be considered when deciding the final strain to select. The results show that, as with biomass productivity, the biochemical composition of the biomass is not altered greatly based on the different culture media assayed, it is mainly a function of the strain used (Fig. 7). The *Nostoc commune* and *Spirulina platensis* strains showed the highest protein content, over 40%d.wt., whereas bloom from the River Seine and *Scenedesmus almeriensis* showed the lowest protein content, below 20%d.wt. (Fig. 7A). In terms of lipids, *Chlorella vulgaris* and *Neochloris oleoabundans* gave the highest values, above 25%d.wt., whereas cyanobacteria such as *Spirulina platensis* and *Anabaena* sp. had the lowest lipid contents, lower than 10% d.wt. (Fig. 7B). Carbohydrates were the major component of most strains, especially high for some strains such as *Scenedesmus almeriensis, Anabaena* sp. and bloom from the River Seine, with values above 60% d.wt., whereas this was far lower for lipid-rich strains such as *Neochloris oleoabundans* and *Chlorella vulgaris*, with values below 40%d.wt. (Fig. 7C). The biochemical composition of any microalgae is greatly dependent on the phase at which the biomass is harvested. In our case, the biomass was collected at the end of the stationary phase, when the protein content is lower but the lipid and carbohydrate contents are higher – depending on the nature of the strain and its strategy for energy storage. Most of the strains accumulate mainly carbohydrates except for *Neochloris oleoabundans* and *Chlorella vulgaris*, both of which mainly accumulate lipids. Many references exist regarding the biochemical composition of the different strains evaluated here but all were performed under different conditions. Thus, the values for the protein, lipid and carbohydrate contents, of 23.0, 14.0 and 60.0%, respectively, were reported for *Chlorella vulgaris* [35], while similar values were also reported for *Chlorella vulgaris* and *Scenedesmus sp.* [36]. The lipid content for *Neochloris oleoabundans* ranged from 14 to 26% d.wt. as a function of the culture conditions - the lipid content increasing as the growth rate was reduced by nitrogen limitation [37]. The different protein, carbohydrate and lipid contents of the produced biomass indicate that each could be used for different purposes in

biofuel production (i.e. biodiesel, bioethanol, biogas), agricultural products (biofertilizers), animal feed (including aquaculture), etc. [38,39]. However, the biomass value varies in the diverse applications so, to achieve a reliable process, it is necessary to identify a target market where the biomass value will be higher than its production cost [10].

To estimate the biomass value (€·kg^{-1}) and thus the economic yield (€·m^{-3}·day^{-1}), we considered both the biomass productivity and the biochemical composition of the produced biomass as well as a fixed value for the main biomass components such as proteins (1.0 €·kg^{-1}), lipids (0.6 €·kg^{-1}) and carbohydrates (0.3 €·kg^{-1}) (Fig. 8). These values were taken from the market value of regular components such as proteins used in animal feed, oils used in biodiesel production and cereals used in bioethanol production; these results can vary depending on the different market values considered. The data show that *Scenedesmus almeriensis*, *Neochloris oleoabundans* and bloom from the River Seine had the highest biomass productivities but the bloom and *Scenedesmus almeriensis* generally gave low values for components such as carbohydrates, up to 0.8 g·L^{-1}·day^{-1}, whereas *Neochloris oleoabundans* produced equal amounts of high value components such as proteins and lipids as low value components such as carbohydrates, around 0.4 g·L^{-1}·day^{-1} (Fig. 8A). Considering the price of diverse biomass components, the results show that the biomass value of the tested strains ranged from 0.15 to 0.68 €·kg^{-1}; being maximal for *Neochloris oleoabundans* and minimal for *Anabaena* sp. (Fig. 8B). By combining the biomass productivity with the biomass value, the economic yield can be calculated - the results show that despite variations in the biomass value, the biomass productivity is the most relevant parameter determining the economic yield, so the most productive stains are the most promising (Fig. 8C). Consequently, *Scenedesmus almeriensis*, *Neochloris oleoabundans* and bloom from the River Seine showed the higher economic yields, above 0.7 €·m^{-3}·day^{-1}, whereas most of the cyanobacteria showed lower economic yields, below 0.2 €·m^{-3}·day^{-1}. These values were lower than the microalgae biomass production cost whatever the microalgae production system used, ranging from 5 to 50 €·kg^{-1}; nonetheless, this can be significantly reduced by combining the microalgae production step with wastewater treatment (sewage, centrate, manure, etc.) and flue gases (CO_2 capture) [40,41]. So, although research and development are still needed to attain commercial processes, the reported values confirm that microalgae can be used for CO_2 capture if adequate systems are developed. Moreover, the coupling of microalgae production with the treatment of wastewaters and flue gases means distributed biomass production close to the effluent treatment sites, thus facilitating the implementation of these types of processes.

3.1. Conclusions

To develop any microalgae-based process, suitable strains must be selected based on the final objective. In the case of CO_2 capture, robust and highly productive strains tolerant to the water quality available at the production site must be used. From the 11 preselected strains, we concluded that all could be produced in Seine river water. This water is not toxic to the selected strains; indeed, it proved to contain the minor nutrients required for biomass production although not the main ones that fertilizers can provide. Out of the strains tested, *Scenedesmus almeriensis*, bloom from the River Seine and *Neochloris oleoabundans* were the most productive, and although the first two contained mainly carbohydrates, and only *Neochloris oleoabundans* had a high lipid content, the biomass value was similar for all of them, as was their economic yield. In any case, the performance of the microalgae tested was clearly higher than that of the cyanobacteria we assayed, meaning that for CO_2 processes, the utilization of this type of microorganism is more advantageous because of its higher productivity and more interesting biochemical composition. Estimations regarding the value of the produced biomass confirm that, given its low market value, the biomass production cost in current production systems is higher than the estimated biomass value. Consequently, to achieve viable processes, they need to be coupled with waste treatment processes.

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