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Treatment of secondary urban wastewater with a low ammonium-tolerant marine microalga using zeolite-based adsorption

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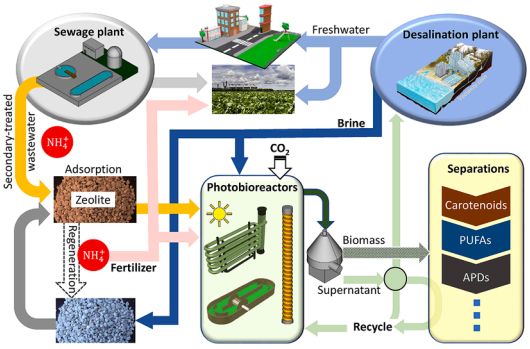
HIGHLIGHTS

- Zeolites provide ammonium-rich wastewater streams tolerable to marine microalgae.
- The ammonium adsorption of the natural zeolite used is among the highest reported.
- *Amphidinium carterae* grows in culture media containing zeolite-treated UWW.
- To adjust the culture medium salinity improves the growth of *A. caterae*.
- Zeolite-treated UWW allow to produce microalga's specialty metabolites sustainability.

GRAPHICAL ABSTRACT



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ABSTRACT

The low tolerance of marine microalgae to ammonium and hyposalinity limits their use in urban wastewater (UWW) treatments. In this study, using the marine microalga *Amphidinium carterae*, it is demonstrated for the first time that this obstacle can be overcome by introducing a zeolite-based adsorption step to obtain a tolerable UWW stream. The maximum ammonium adsorption capacities measured in the natural zeolite used are among the highest reported. The microalga grows satisfactorily in mixtures of zeolite-treated UWW and seawater at a wide range of proportions, both with and without adjusting the salinity, as long as the ammonium concentration is below the threshold tolerated by the microalgae (6.3 mg L^{-1}). A proof of concept performed in 10-L bubble column photobioreactors with different culture strategies, including medium recycling, showed an enhanced biomass yield relative to a control with no UWW. No noticeable effect was observed on the production of specialty metabolites.

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1. Introduction

Microalgae-based wastewater treatments (MBWTs) have received a lot of attention in the last few years. Their greater potential for efficiently recovering nutrients compared to well-established wastewater treatment processes has recently been reviewed (Li et al., 2019; Liu et al., 2020). Most of the studies focused on the three typical streams originating in urban wastewater (UWW) plants: raw sewage, secondary effluent and centrate. UWW streams contain variable concentrations of different nitrogen (e.g. ammonium, nitrate, urea, etc.) and phosphorus (e.g. phosphates) sources, which serve as macronutrients for most microalgae (Li et al., 2019). Consequently, the nutrient profiles and physical properties of these sources vary significantly, as does the microalgae's adaptability to different types of UWW streams; the species are also strongly dependent on the local climate. Although ammonium is the preferred nitrogen form for microalgae, it is toxic at relatively low concentrations (Collos and Harrison, 2014). Therefore, the microalgae's tolerance level to this nutrient is of critical importance when considering the MBWT because ammonium is present in all UWWs. Even so, many microalgae species have been studied for use in MBWT (Li et al., 2019; Liu et al., 2020); the vast majority have been freshwater species although a few marine species have been investigated.

Different technologies for ammonium recovery from domestic wastewaters have emerged as alternative options (Cruz et al., 2019). Prominent among these are the adsorption-based processes using low-cost industrial adsorbents that are environmentally sustainable and recyclable (Han et al., 2021). Natural zeolites, whether chemically treated or not, stand out as the main candidates compared to other alternatives, as can be found in detailed and comprehensive tables and information reported in recent studies (Cruz et al., 2019; Han et al., 2021; Montalvo et al., 2020; Muscarella et al., 2021; Wang and Peng, 2010). Natural zeolites are highly porous aluminosilicate minerals that exhibit high cation-adsorption performance in aqueous solutions containing ammonium and heavy metals (Wang and Peng, 2010). Although zeolites are less efficient at adsorbing anionic ions (nitrates, phosphates and sulphates, etc.) and organics that are also present in wastewaters, modifying their forms can eliminate this problem (Wang and Peng, 2010).

The use of microalgae that have a low tolerance to ammonium and/or other typical wastewater pollutants could be significantly improved if the MBWT were combined with a prior zeolite-based adsorption step to obtain a feed stream tolerated by the selected microalgae, as reported in a few studies carried out with freshwater microalgae (Markou et al., 2014; Lu et al., 2019), but not for marine species. In these studies, zeolite was used for wastewater ammonia adsorption in a first step and, subsequently, the ammonia-enriched zeolite was added to the culture medium to slowly release ammonia into it. This strategy was not without problems, such as biofouling formation on the zeolite surface (Markou et al., 2014). The biofouling that develops on the surfaces of culture devices becomes a source of ongoing infection, unless it is fully removed and the exposed surfaces thoroughly cleaned (Molina-Grima et al., 2021).

Given that marine dinoflagellate microalgae can provide numerous unique and potentially useful bioactives (Assunção et al., 2017; Gallardo-Rodríguez et al., 2012), biomass biorefining from this group of microalgae has been proposed in recent years (López-Rodríguez et al., 2019; López-Rodríguez et al., 2021; López-Rosales et al., 2018). However, compared to the most studied microalgae classes (Chlorophyceae, Cyanophyceae, Prymnesiophyceae, Diatomophyceae, and Raphidophyceae) (Collos and Harrison, 2014), dinoflagellates are the least tolerant to ammonium. Therefore, it is not surprising that dinoflagellate microalgae have been largely unexplored in terms of MBWT. To the best of our knowledge, only one study has been carried out in which a marine dinoflagellate was cultured in low proportions (10 mL culture volumes) of local municipal wastewater (Ho et al., 2013), reporting satisfactory results. Hence, it is important that this particular microalgae group is thoroughly investigated.

The dinoflagellate microalga *Amphidinium carterae* is a good model system to use in MBWT studies since this species can best represent the feasibility of a sustainable dinoflagellate-based bioprocess. *A. carterae* produces biomass that is rich in high added-value metabolites such as amphidinols (APDs), carotenoids and PUFAS (Abreu et al., 2019; Fuentes-Grunewald et al., 2016; López-Rodríguez et al., 2021; Molina-Miras et al., 2018) and can be successfully cultured in aquaculture effluents containing different nitrogen sources, including ammonium (Molina-Miras et al., 2020a). The aim of the work presented here therefore is to investigate how well this marine microalga can grow in urban-wastewater-based culture media, whether treated, or not, with a zeolite-based adsorption step to reduce its ammonium concentration below the threshold tolerated by *A. carterae*, both with and without adjusting the salinity, and to determine the equilibrium adsorption capacity of the zeolites in this wastewater. The experiments were carried with wastewater from secondary effluent produced following activated sludge treatment in an aeration tank. The effect of this treatment on the biomass composition of high-value microalgal metabolites such as PUFAs, carotenoids and amphidinols was evaluated with one the best medium in 10-L bubble column photobioreactors.

2. Materials and methods

2.1. Urban wastewater characterization.

Secondary-treated urban wastewater was obtained from the "Bobar" sewage treatment plant in Almería, Spain (Lat: 36.823495; Long: −2.422471; population 245,000). The plant is operated by the company Aqualia S.A. (Spain). The composition of the secondary-treated wastewater was: 16.00 ± 0.59 mg L^{-1} biological oxygen demand (BOD_5); 87.00 ± 2.8 mg L^{-1} chemical oxygen demand (COD); 37.0 ± 12.3 mg L^{-1} total suspended solids; 6.00 ± 0.23 mg L^{-1} sedimentable solids (SS); 56.9 mg L^{-1} NH_4^+-N; 2.1 mg L^{-1} NO_3^--N; 0.78 mg L^{-1} PO_4^{-3}-P; 7.26 ± 0.11 pH; and 1.4 PSU salinity. To prevent biological contamination and any residual solids interfering with the adsorption tests, the wastewater (henceforth designated as UWW) was filter-sterilized (0.22 μm Millipore filter; Millipore Corporation, Billerica, MA, USA) prior to use. As described in the sections below, both the UWW obtained in this way and the UWW treated using natural zeolites (to reduce the ammonium concentration) were mixed with seawater in different proportions to prepare the culture media.

2.2. Adsorbent characterization

A commercial natural zeolite (ref. ZE 700, Prodac International, Cittadella, Italy) with a specific surface area of around 600 m^2g^{-1} was used in this study. A total of 500 g of zeolite was ground in a ceramic ball mill. The experimental mill, comprising 25 ceramic balls (27.5 mm diameter and weighing 27 g each), was laboratory scale (190 mm diameter, 190 mm length), and provided a total mill volume of 3.94 L; the balls accounted for 7% of the mill volume. The mill's rotation speed was 168 rpm. After 360 min of grinding, the sample was transferred to a sieve shaker (model FT-200 M, Filtra Vibración, Barcelona, Spain) equipped with six sieves arranged sequentially from the largest downwards (according to their average mesh size). The sequence was as follows: 4.00, 1.00, 0.50, 0.3, 0.1, and 0.05 mm. After 10 min of shaking at a power rate of 9 W, three fractions were obtained with the following particle diameter ranges: F1 (powder, 0.05–0.10 mm), F2 (coarse, 0.3–0.5 mm) and F3 (pebble, 1.00–4.00 mm). The different fractions were initially washed with deionized water to remove any non-adhesive impurities, and then dried in an oven at 100 °C for 24 h.

Two F3 samples (115 g each) were used to identify the zeolites' mineral species by means of X-ray diffraction (XRD, Bruker-D8 Advance equipment with DaVinci geometry, USA). The data were analysed using

the Diffrac.Suite™ software package (Bruker, USA). Heulandite-Na (84.23 ± 0.01%) was found to be the main component although small amounts of other crystalline phases such as cristobalite (7.75 ± 0.35%), anorthite (3.77 ± 0.08%), mica (3.69 ± 0.38%), and rutile (0.56 ± 0.06%) were also detected. It is well known that heulandite-type zeolites are amongst the most abundant and useful zeolites found in nature.

The chemical composition of the samples was determined by X-Ray fluorescence (XRF) in a wavelength-dispersive X-ray fluorescence spectrometer (model S4 Pioneer, Bruker, USA). The data analysis was carried out using the SPECTRA^{plus} software package (Bruker, USA), which provided the following mean percentage contents of the studied samples, ordered from highest to lowest: SiO_2 (64.083 ± 0.646), Al_2O_3 (10.693 ± 0.155), K_2O (3.524 ± 0.019), CaO (2.884 ± 0.025), Fe_2O_3 (1.449 ± 0.019), MgO (0.606 ± 0.012), Na_2O (0.395 ± 0.015), TiO_2 (0.154 ± 0.003), BaO (0.122 ± 0.008), SrO (0.041 ± 0.001), MnO (0.022 ± 0.002), P_2O_5 (0.022 ± 0.002), SO_3 (0.019 ± 0.002), Cl (0.015 ± 0.003), ZrO_2 (0.015 ± 0.000), Rb_2O (0.014 ± 0.001), Y_2O_3(0.002 ± 0.000). The results revealed the predominance of SiO_2 and Al_2O_3, and to a lesser extent K_2O, CaO, Fe_2O_3, MgO and Na_2O; the rest of them were in trace concentrations. A chemical composition such as this is in line with those determined worldwide for natural zeolites (Wang and Peng, 2010). The values of the SiO_2/Al_2O_3 (=5.993) and $(K_2O + Na_2O)/(MgO + CaO)$ (=1.223) ratios were consistent with those reported as indicative of zeolites being present (4.27–6.48 and 0.76–1.53, respectively) (Ostrooumov et al., 2012).

2.3. Batch study for ammonium removal from the UWW using zeolites.

The equilibrium adsorption of the three average particle sizes (F1 to F3) was studied using different initial concentrations of ammonium in the secondary-treated UWW. To do this, UWW with a constitutive $NH_4^+ - N$ concentration of 56.9 ± 3.7 mg L^{-1} (see above) was implemented with NH_4Cl. Initial $NH_4^+ - N$ concentrations below 56.9 mg L^{-1} were achieved by adjusting the pH to 11.5 with NaOH, followed by simple stripping at room temperature with subsequent HCl neutralization. A total of eight UWWs with $NH_4^+ - N$ concentrations of 0, 27, 57, 117, 272, 428, 1167 and 2333 mg/L were prepared. The experiments were carried out in batch mode by adding 2 g of zeolite to 200 mL of UWW in 500-mL Pyrex™ borosilicate glass Erlenmeyer flasks, equivalent to a 10 g/L concentration, as reported earlier (Huang et al., 2010). The flasks were agitated on an orbital shaker (3-cm shaking diameter) at a shaking frequency of 120 rpm for 24 h. During the experiments, 2 mL samples were taken every two hours for the first 8 h, plus a sample at the end of the experiment. Sampling was carried out without stopping the agitation to prevent particle sedimentation and to maintain homogeneous zeolite particle suspension in the flasks. Each sample was then centrifuged to recover the clean supernatant from the zeolite pellets. The supernatants were stored at −20 °C for further NH_4^+ analysis. All the experiments were duplicated at a temperature of 21 °C and a pH of 8; from these, the average value was used.

From the NH_4^+-N concentration measurements in the supernatants taken over time, the zeolite's NH_4^+ removal efficiency (*E*%) and NH_4^+-N adsorption capacity at time *t* (q_t, mg NH_4^+ /g zeolite) were calculated as follows (Huang et al., 2010):

$$E\,\% = \frac{C_0 - C_t}{C_0} \cdot 100$$

$$q_t = \frac{(C_0 - C_t)\cdot V}{m}$$

where *V* is the wastewater sample volume (L); *m* is the mass of adsorbent (g zeolite); C_0 is the initial ammonium concentration in the solution (mg NH_4^+-N/L); and C_t is the ammonium concentration in the solution at time *t*. The NH_4^+ adsorption capacity at equilibrium, (q_e, mg NH_4^+/g zeolite) was determined by the equation:

$$q_e = \frac{(C_0 - C_e)\cdot V}{m}$$

where C_e is the ammonium concentration in the solution (mg NH_4^+-N/L) once equilibrium had been reached (data obtained at 24 h).

The interaction of ammonium ions with the energetic adsorption sites on the zeolite surface was analyzed using the Langmuir and Freundlich isotherms given by the following equations (Huang et al., 2010):

$$q_e = \frac{q_{\max} K_L C_e}{1 + K_L C_e}$$

$$q_e = K_F C_e^{1/n}$$

where q_{max} (mg NH_4^+/g) and K_L (L/mg) are the maximum ammonium adsorption capacity and the equilibrium constant, respectively. K_F and $1/n$ represent the Freundlich capacity coefficient and the Freundlich intensity parameter, respectively.

2.4. Microalga and culture experiments

The marine dinoflagellate microalga *Amphidinium carterae* Dn241EHU (Culture Collection of the Plant Biology and Ecology Department at the University of the Basque Country) was used. The inoculum and experiments were cultured in T-flasks (Nunc EasyFlask 175 cm^2, Fisher Scientific SL, Madrid, Spain) with a 0.4 L working volume at 21 ± 1 °C under a 12/12 h light–dark cycle. The lighting system consisted of light emission diode (LED) strips, the spectral profile of which is described elsewhere (López-Rosales et al., 2016); these were attached to a rectangular-shaped flat reflective plastic cover, and the T-flasks were arranged vertically in front of them (Molina-Miras et al., 2020a). The mean PAR irradiance measured on the T-flask surface facing the LEDs was 300 μmol photons·m^{-2}·s^{-1}. The inoculum was pre-acclimated to these environmental conditions.

The f/2 medium formulation (Guillard, 1975), modified to have a N/P ratio of 5 by increasing the P concentration (Molina-Miras et al., 2018), was used both for inoculum maintenance and for the experiments when required. All the culture media were filter-sterilized (0.22 μm filtration, Thermo Scientific Nalgene Rapid-Flow™, Fisher Scientific SL, Madrid, Spain). The experiments were conducted in static batch cultures starting at a mean initial biomass concentration of 11 ± 1 mg L^{-1} (approximately equivalent to a cell concentration of 6.9 10^4 cell ml^{-1}).

The zeolite-treated UWW used in the experimental plan was prepared in two ways. To reduce the ammonium concentration, the first had the protocol described above (Section 2.3) applied to the raw UWW using zeolite powder (fraction F1, 0.05–0.10 mm). Briefly, the zeolite powder was washed with deionized water and then dried. Next, 2 g of the dried zeolite powder was added to 200 mL of raw UWW, equivalent to a 10 g/L concentration. After the necessary contact time, the mixture was centrifuged to recover the clean supernatant (i.e. the zeolite-treated UWW) from the zeolite pellets. The resulting composition of the zeolite-treated UWW was as follows: 9.25 ± 0.07 mg L^{-1} NH_4^+-N; 0.68 ± 0.05 mg L^{-1} NO_3^--N; 0.063 ± 0.009 mg L^{-1} PO_4^{-3}-P. Therefore, the N and P concentrations were markedly lower, close to 85% for NH_4^+-N and at trace levels for the rest. In the second procedure, the UWW was treated with a zeolite concentration (30 gL^{-1}) sufficient to completely remove the ammonia, nitrates, and phosphates.

The influence on the growth and biomass yield of *A. carterae* of the different culture media prepared using Mediterranean seawater, UWW, the zeolite treatment, and salinity adjustment was also investigated. All the prepared culture media are described in detail in Table 1. As one can observe, both the UWW and the seawater were used as the basis for the culture medium after being treated with zeolite (coded with Z for UWW) or left untreated. The zeolite-treated UWW was mixed with seawater in different proportions (0, 25, 50, 75% and 100% v/v) and tested, both by

Table 1
Summary of the experimental design assayed to assess the tolerance of the marine microalga *A. carterae* to different culture media prepared from secondary-treated urban wastewater, treated with zeolite (Z) or untreated (UWW), at different wastewater proportions (0, 25, 50, 75, and 100), with (S) or without the salinity adjusted. The nitrogen and phosphorus concentrations correspond to those established in the culture medium at the beginning of each culture. The f/2 medium formulation was used for the experiments with added nutrients.

Characteristics	Culture media														
	CTLO	CTL1	CTL2	CTL3	CTL4	ZS25	Z25	ZS50	Z50	ZS75	Z75	ZS100	Z100	UWWS	UWW
UWW (%)	–	–	–	–	–	–	–	–	–	–	–	–	–	100	100
Zeolite-treated UWW (%)	–	–	100	50	–	25	25	50	50	75	75	100	100	–	–
Seawater (%)	100	100	–	50	–	75	75	50	50	25	25	–	–	–	–
Zeolite-treated SW (%)	–	–	–	–	100	–	–	–	–	–	–	–	–	–	–
Salinity Adjustment	No	No	Yes	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Salinity (‰)	38.0	38.0	38.0	38.0	38.0	38.0	28.9	38.0	19.7	38.0	10.6	38.0	1.4	38.0	1.4
$[NH_4^+\text{-N}]_o$, mg L^{-1}	0.0	0.0	0.0	0.0	0	2.3	2.3	4.6	4.6	7.0	7.0	9.3	9.3	54.4	54.4
$[NO_3^-\text{-N}]_o$, mg L^{-1}	12.3	0.0	0.0	0.0	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3	2.1	2.1
N_{T0}, mg L^{-1}	12.3	0.0	0.0	0.0	12.3	14.6	14.6	16.9	16.9	19.3	19.3	21.6	21.6	56.5	56.5
$[PO_4^{-3}\text{-P}]_o$, mg L^{-1}	5.4	0.0	0.0	0.0	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	0.8	0.8
$[TOC]_o$, mg L^{-1}	11.2	10.8	23.2	17	13.9	14.6	14.6	18	18	21.5	21.5	24.9	24.9	31.6	31.6

adjusting the salinity to that of seawater (coded as CTR0, ZS25 to ZS100) and without adjusting the salinity (i.e., with that resulting from the mixtures coded as CTR0, Z25 to Z100). The culture media were enriched with f/2 nutrient formulation. The salinity adjustment was carried out with a brine prepared from sea salts. To decouple the effects in the results analysis, five control cultures, coded as CTL0-4, were included: CTL0 consisted of the f/2 medium prepared with seawater; CTL1 consisted of the untreated seawater without f/2 nutrients; CTL2 consisted of the UWW treated with zeolite until the ammonia, nitrates, and phosphates were completely removed, and without f/2 nutrients; CTL3 consisted of a mixture of 50% CTL1 and 50% CTL2; and CTL4 consisted of the f/2 medium prepared with zeolite-treated seawater. The assays, summarized in Table 1, were carried out using cells acclimated to each culture medium, except in the cases where cell death occurred. The cultures were inoculated with cells that were in linear growth phase. Each experiment was conducted in duplicate. The initial pH for all the cultures was fixed at 8 using 0.1 M HCl or 0.1 M NaOH. Since only around 10% of the total ammonia is present as ammonia (NH_3) at that pH, close to 90% is present as NH_4^+, thus the nitrogen from ammonium would be indistinctly referred to as NH_4^+-N, even though both ammonia and ammonium were present at the initial fixed pH. The pH was allowed to evolve freely in all the cultures. The experimental plan allowed us to assess the effects of: (i) the f/2 medium composition when prepared in untreated seawater (comparing the experiments coded as CTL0 and CTL1); (ii) the treatment of UWW with zeolite but without f/2 nutrients (comparing the experiments coded as CTL1, CTL2 and CTL3); (iii) the zeolite treatment using seawater with f/2 nutrients (comparing the experiments coded as CTL0 and CTL4); (iv) the complete removal of ammonia, nitrates and phosphates contained in the UWW using zeolite (comparing the experiments coded as CTL2 and ZS100); (v) the salinity using UWW 100% without f/2 nutrients (comparing the experiments coded as UWW and UWWS) and the zeolite-treated UWW enriched with f/2 nutrients (comparing the experiments coded as Z100 and ZS100); and (vi) different proportions of zeolite-treated UWW enriched with f/2 nutrients, both with and without adjusting the salinity (comparing the experiments coded as CTL0, Z25, ZS25, Z50, ZS50, Z75, ZS75, Z100, and ZS100). All the experiments were duplicated.

2.5. Culture in 10-L bubble-column photobioreactors

The culture media coded ZS50 in Table 1 was selected for the proof of concept in the photobioreactors (PBR); this was because it had provided satisfactory results (see the results below) following the experiments carried out in Section 2.4. Thus, a mixture of zeolite-treated UWW and seawater at a 50% (v/v) proportion, with its salinity adjusted to 38‰, was used as the basis for the culture medium (the experiment coded ZS50-PBR). In order to improve the biomass yield of the PBR cultures compared to the T-flask cultures, three-times the f/2 nutrient concentration was added (i.e., f/2 × 3), apart from the sodium orthophosphate, which was increased 15-fold to achieve a nitrogen-to-phosphorus ratio of 5. (N/P = 5). The culture medium of the control culture was prepared by substituting the zeolite-treated UWW with seawater (the experiment coded CTR-PBR).

The photobioreactors consisted of 10 L culture volumes (0.09 m internal diameter, 1.75 m high) in vertically positioned poly(methyl methacrylate) bubble columns. The cultures were agitated by air sparging at a flow rate of 4 L min^{-1} (i.e., 0.4 vvmin), with air filtered from a compressor being injected through a 2-mm nozzle sparger placed at the bottom of the PBR. Illumination was provided by multicolored LED strips (red, green, blue, and warm white, collectively referred to as RGBWW; Edison Opto Co., Taiwan) wound in a spiral around the PBRs. The irradiance at the center of the PBRs (filled with seawater), measured using a QSL-100 quantum scalar irradiance sensor (Biospherical Instruments, San Diego, USA), was 200 μmol photons·m^{-2}·s^{-1}. A 12 h/12 h light/dark (L/D) cycle was employed. The culture temperature was controlled at 20 ± 1 °C and the pH was controlled at pH 8.5 by automatically injecting carbon dioxide, as required. The PBR sterilization and cleaning procedures were carried out as described previously for a similar characteristic PBR but at a pilot-scale size at which dinoflagellate microalgae had already been cultured (López-Rosales et al., 2016).

The sequential-batch cultivation mode was used to explore the potential of culturing *A. carterae* in ZS50 medium. To do this, the PBR cultures started with an initial batch culture phase in which the culture medium (9 L) was inoculated with 1 L of an inoculum containing cells in the late exponential growth phase of a culture in ZS50 medium (coded as ZS50-PBR-1). Once the stationary growth phase was reached, a second batch culture was initiated by removing a variable culture volume and replenishing it with an equal volume of fresh medium (coded as ZS50-PBR-2). After measuring the phosphate, nitrate and ammonium in the supernatant, the fresh medium was supplemented with stock solutions of the three nutrients so that their final concentrations in the whole culture volume (=10 L) were close to the values established for the ZS50 and control media. The other remaining nutrients were added in equivalent quantities to those of the selected medium formulation. After each replenishment, a biomass concentration of about 0.080 ± 0.014 g L^{-1} d.w. was set as a baseline for all the batch cultures. The harvested culture volume from the second batch culture was centrifuged at 5000 × g for 5 min at room temperature in a benchtop centrifuge (Beckman Coulter, model Allegra 25R, Madrid, Spain). These centrifugation conditions were within the operating window that guarantees maximum cell recovery and cell integrity for *Amphidinium carterae* (Molina-Miras et al., 2019). The experiments were duplicated.

Additionally, in a last batch culture (coded as ZS50-PBR-2R), the effect of recycling the cell-free supernatant on the growth kinetics and the production of interesting metabolites was assessed. This was prepared as follows: the exhausted supernatant from the second batch

culture was filtered through a 0.2 μm filter (Sartorius Stedim Biotech; model Sartopore 2 Sterile Midicap) and then autoclaved. Thus, a certain volume of the second batch culture was replaced with the exhausted supernatant replenished with nutrients, in a similar way to that carried out in the second batch. These experiments were duplicated. The maximum photochemical yield of photosystem II (F_V/F_M) was determined in all the PBR cultures using a pulse amplitude modulation (PAM) chlorophyll fluorometer (Mini-PAM-2500; Heinz Walz GmbH, Germany).

2.6. Growth kinetic parameters

The dimensionless biomass concentration (C/C_o) versus time (t) data were fitted to the logistic equation reformulated to obtain parameters with a biological meaning (Zwietering et al., 1991):

$$Ln\left(\frac{C}{C_o}\right) = \frac{A}{1+\exp\left[\frac{4\mu_m}{A}(\lambda - t)+2\right]}$$

where the three growth curve phases of a batch culture are described by three kinetic parameters: the maximum specific growth rate, μ_m; the lag time, λ; and the asymptote [$A = \ln(C_\infty/C_o)$], which is the maximal value reached. The deconvolution of equation provides the evolution of biomass concentration. This latter term was used to calculate the theoretical global biomass productivity, P_b, at any culture time, in the following way:

$$P_b(t) = \frac{C(t) - C_o}{t}$$

with eq. providing the maximum value, P_{bmax}.

2.7. Analytical measurements

A conductivity sensor was used to measure the salinity in the culture media. The Nessler method was employed to quantify the amount of ammoniacal nitrogen present in the different experiments performed (ASTM International, 2014). The phosphate-P and nitrate-N in the supernatants were determined as described in a recent study (Molina-Miras et al., 2018). For this, the American Public Health Association methods 4500-P and 4500-N were followed (APHA et al., 2012). The measurements were carried out in triplicate using a multiplate spectrophotometer (BioTek Synergy ™ Mx; Swindon, United Kingdom).

The amphidinols (APDs) were determined through RMN analysis from methanolic extracts of the harvested biomass, as described elsewhere (Abreu et al., 2019). These measurements were duplicated. The carotenoids were determined using an HPLC system with a photodiode array detector, as explained elsewhere (López-Rodríguez et al., 2020). The HPLC calibration was performed using the external standards provided by DHI (carotenoids peridinin, β-carotene, diatoxanthin, dinoxanthin and diadinoxanthin). The molar extinction coefficients reported in the literature were used to quantify those carotenoids not calibrated using the commercial standards. All the analyzed samples contained the same carotenoid pigments determined in previous studies on *A. cartereae* (López-Rodríguez et al., 2019; Molina-Miras et al., 2018): peridinin, peridininol, diadinochrome, β-carotene, diatoxanthin, dinoxanthin, and diadinoxanthin. The fatty acid methyl esters (FAMEs) content and profile were determined by direct transesterification, as described previously (Rodríguez-Ruiz et al., 1998). The samples were analyzed in a gas chromatograph coupled to a flame ionization detector (FID) (Agilent Technologies 6890 N Series Gas Chromatograph, Santa Clara, CA, USA). All the analyzed samples contained the same PUFAs as previously identified in studies on *A. cartereae* (Molina-Miras et al., 2018): linoleic acid (18:2n6), 18:3n3, stearidonic acid (SDA; 18:4n3), arachidonic acid (20:4n6), eicosapentaenoic acid (EPA; 20:5n3) and docosahexaenoic acid (DHA; 22:6n3). The measurements were carried out on duplicate samples.

2.8. Statistical analyses

One-way and two-way analysis of variance (ANOVA) tests were used for the significant difference analysis. Statistically significant differences in the mean response between factors were fixed at a 5.0% significance level threshold ($p < 0.05$). Fisher's least significant difference (LSD) procedure was the method used to discriminate between the means at the 95.0% confidence level. The "replicate" factor did not have a statistically significant effect. Statistical data analyses were performed using the Statgraphics Centurion XVII (version 17.2.04) statistical software (2014, Statpoint Technologies, Inc., Warrenton, VA).

3. Results and discussion

3.1. The NH_4^+ removal capacity of the natural zeolite

The percentages of the removed ammonium (E%) from the UWW for each of the three zeolite sizes were measured at different initial NH_4^+-N concentrations (C_o) once equilibrium was reached (see Fig. 1A). The particle size and C_o had a statistically significant effect on the E% ($p < 0.05$ in the two-way ANOVA). Fig. 1A shows that, as the C_o increased, the E% decreased; this is the typical response for an ion-exchange process that becomes progressively saturated with an increasing adsorbate concentration in the solution, involving an increase in the driving force required for the further exchange of NH_4^+ ions onto the zeolite (Huang et al., 2010). In addition, the NH_4^+ removal efficiency increased as the particle size decreased ($p < 0.05$) because the size reduction led to a larger surface area.

The two most widely used ion-exchange isotherms, the Langmuir and Freundlich equations, were used to characterize the equilibrium relationship q_e versus C_e. The equilibrium experimental data and the result of fitting the isotherms to them are shown in Fig. 1B. It is evident that the Langmuir model had a better fit than the Freundlich model over the three particle size ranges (the determination coefficients are not shown for the sake of clarity). The effect of particle size on the Langmuir model parameters is represented in Fig. 1C. The maximum adsorption capacities (q_{max}) were inversely related to the particle size ($p < 0.05$). This trend is supported by a recent study in which q_{max} data from various natural zeolites were comprehensively reviewed (Putra and Lee, 2020). This particle-size dependency might be related to the estimated binding energy (K_L values) - the smaller the q_{max} value, the higher the K_L value. Another explanation is the porous structure and continuity of the sorbent, namely, that the adsorption of relatively low porosity sorbents is enhanced by reducing the particle size (Liu and Lo, 2001). The q_{max} values in Fig. 1C (49.8 to 21.9 mg NH_4^+ L^{-1}) are among the highest reported in the literature for natural zeolites (Putra and Lee, 2020; Wang and Peng, 2010).

In accordance with the results obtained, the zeolite powder (fraction F1, 0.05–0.10 mm) was chosen for the UWW treatment in the culture experiments (see above). This selection does not imply that this is the optimal particle diameter recommended for a hypothetical industrial-scale application. The characteristic granular, sand-like structure of zeolites restricts the use of full-scale technologies, the conventional packed-bed column being the one most indicated (Cruz et al., 2019). The shape and size of the particles affect the pressure drop in packed-bed columns and, consequently, the hydraulic capacity; however, the optimal ammonium exchange capacity of packed-bed columns also depends on critical properties of the zeolite particles such as their mechanical resistance (Guida et al., 2020). Other variables can significantly affect the selection of the particle size such as the particle size to column diameter ratio, the particle size to bed height ratio or the flow through the column (Medvidović et al., 2006).

3.2. Effects of the culture media on growth kinetics

Fig. 2 shows semilog plots of the normalized biomass concentration

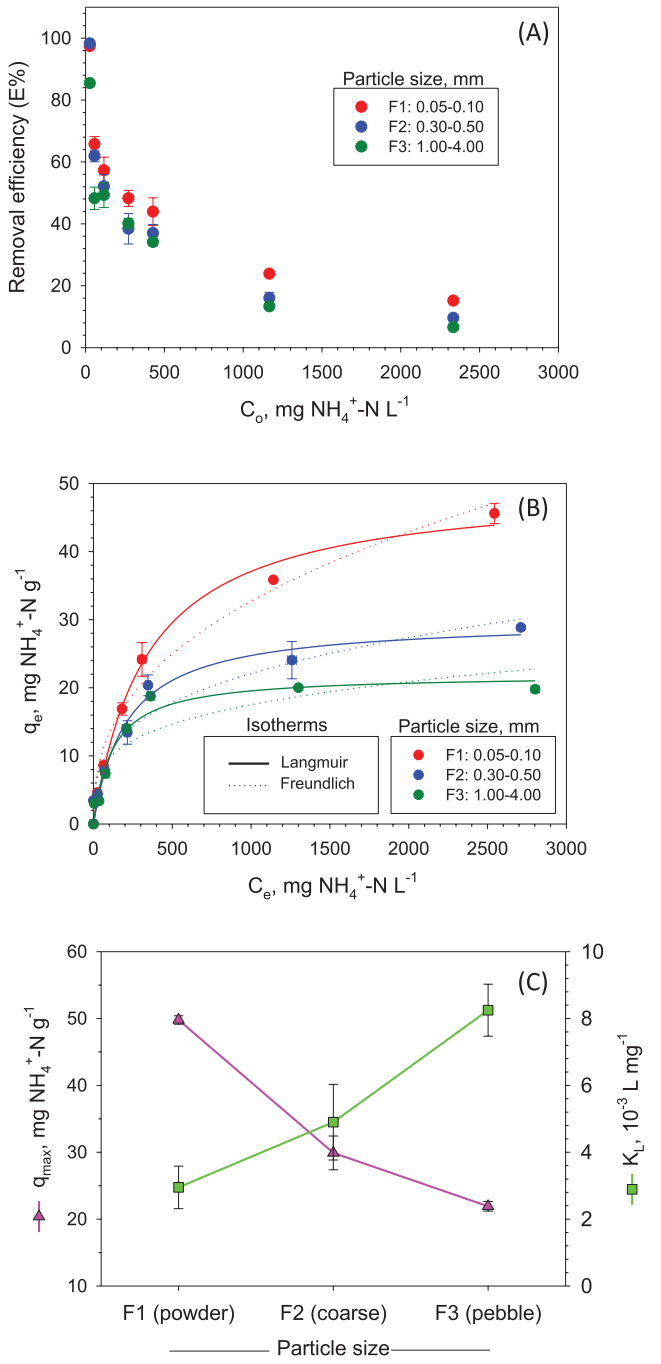

Fig. 1. NH_4^+ removal capacity of the zeolite. **(A)** Effect of the initial ammonium concentration on the NH_4^+ removal efficiency (*E%*) of the three zeolite particle sizes used (F1 to F3). **(B)** Plot of the Langmuir and Freundlich isotherms for the NH_4^+ ion adsorption. Data points are averaged values, and vertical and horizontal bars are the standard deviation for two independent experiments. Data points are averages, and the vertical bars are standard deviations (SD) for samples from duplicate cultures.

for the different treatments described in Table 1. The experimental data are represented by the average values for each duplicate culture treatment. The comparison between CTL0 and CTL1 (Fig. 2A) confirmed that seawater, by itself, without f/2 nutrients (CTL1), could not support cell growth or even satisfy the minimum nutritional requirements for cell maintenance; indeed, the endogenous metabolism went into a marked decline phase after the fifth day of culture. Similar behaviour was observed in the control cultures CTL2 and CTL3 (see Fig. 2B) in which neither nitrogen nor phosphorus were available for the cells. Compared to CTL1, the treatment of UWW with zeolite in CTL2 and CLT3 was not the cause of the decline phase observed, but rather the absence of essential nutrients such as N and P (Fig. 2B). It seems clear that the zeolite treatment was harmless to the cells. This is also supported when comparing the CTL0 and CTL4 experiments (see Fig. 2C), where both growth curves were identical, regardless of whether the seawater was treated CTL4) or not (CTL0) with zeolite before adding the f/2 nutrients.

The zeolite-treated UWW with adjusted salinity (ZS100 in Fig. 2D) had a detrimental effect on growth; no living cells were observed beyond the fourth day of culture. Comparing the ZS100 composition with those of CTL2 (Fig. 2D) and CTL0 (Fig. 2A) displayed in Table 1, it can be concluded that the origin of the toxicity observed in ZS100 might be associated with the initial ammonia concentration (9.3 mg NH_4^+-N L^{-1}); 6.3 mg NH_4^+-N L^{-1} has been reported as the threshold tolerated by *A. carterae* Dn241EHU (Molina-Miras et al., 2020a). This is in line with the results obtained for raw UWW, both with (UWWS) and without (UWW) adjusting the salinity, at ammonium concentrations well above the threshold value tolerated by the microalga (see Fig. 2E). Since the initial ammonium concentration was much higher in the raw UWW than in the zeolite-treated UWW (ZS100), its toxic effect was conspicuously greater. The cells did not survive beyond the first day for the UWW or the third day for the UWWS, indicating that the salinity adjustment somewhat mitigated the detrimental effect of the ammonia. A similar benefit from adjusting the salinity was elicited in the zeolite-treated UWW treatment enriched with f/2 nutrients (ZS100) compared to its counterpart experiment where there was no salinity adjustment (Z100) (Fig. 2F).

The remaining treatments were devised to approximately determine the effect of different Z100:CTL0 (v/v) and ZS100:CTL0 (v/v) ratios (the experiments from Z25 to Z100 and from ZS25 to ZS100, respectively). The experimental results are represented in Fig. 2G-H as points accompanied by lines that represent the corresponding regressions of the experimental data to Eq.. As can be seen in Fig. 2G-H, three proportions (25%, 50% and 75%) permitted microalgal growth. To the best of our knowledge, this is the first time that growth supported by zeolite-treated UWW has been reported for marine microalgae. The non-linear regression analyses of Eq. provided regression coefficient (R^2) values closer to 1 (see the Supplementary Material section), indicating that the logistic growth model provides an excellent description of the experimental data. The variation between the culture media regarding their capacity to promote and sustain growth was established in terms of the kinetic parameters, derived from Eq., and the maximum biomass productivity, P_{bmax}, derived from Eq.. Thus, a one-way ANOVA was carried out for each parameter. As expected, the culture medium had a statistically significant influence on the length of the lag phase in the cultures, λ, (F-ratio = 44.5, p = 0.0.001). The occurrence of lag phases in microalgae acclimated to NH_4^+-N has previously been reported (Molina-Miras et al., 2020a; Przytocka-Jusiak et al., 1977). Since λ was relatively short (the values ranged between 1.1 and 3.2 days), it had no impact on the other parameters. No statistically significant difference exists between the means of *A* (F-ratio = 0.58, p = 0.717) and μ_{max} (F-ratio = 3.16, p = 0.0968). *A* is related to the culture's biomass yield, so similar *A* values are expected because the nitrates and phosphates were not depleted in any of the cultures, with the final mean concentrations being 444 ± 56 μM and 25 ± 4 μM, respectively. The ammonium was completely exhausted, confirming the preference of *A. carterae* in taking up NH_4^+-N at subtoxic concentrations in the presence of other different N sources; this response is common in microalgae (Dagenais-Bellefeuille and Morse, 2013; Lu et al., 2019).

Regarding μ_{max}, Fig. 3A illustrates the result of the corresponding ANOVA multiple range test. The ZS75 medium (i.e. a ZS100:CTL0 ratio of 75%) provided the highest μ_{max} value ($p < 0.05$), being responsible for four pairs of means that showed statistically significant differences. There was no difference between ZS50 and ZS75 ($p > 0.05$). The Z100:CTL0 and ZS100:CTL0 ratios below 75% did not affect the μ_{max} relative to the CTL0 control. Since the main differences between the two culture

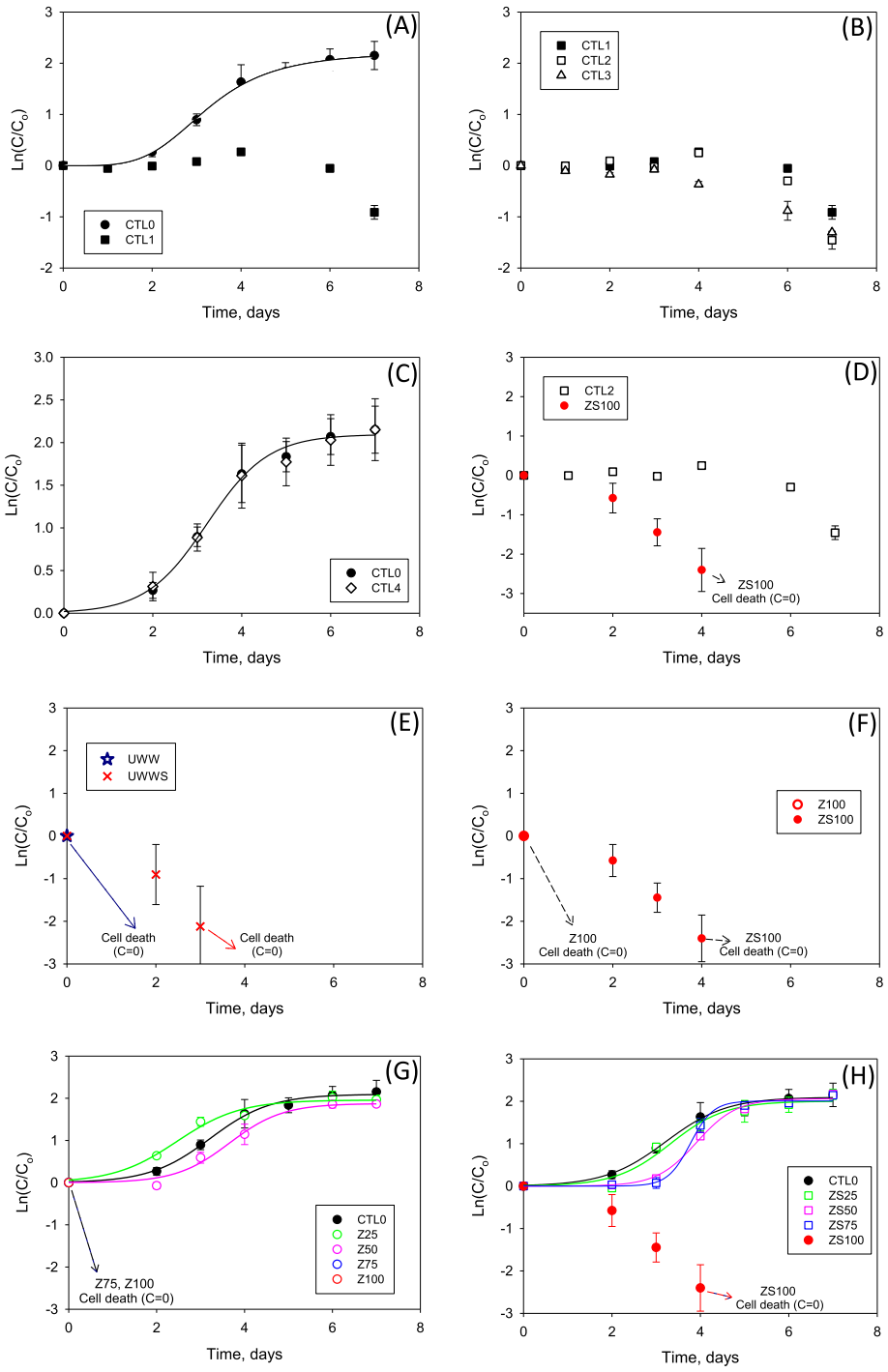

Fig. 2. Comparision of the different culture media described in Table 1 in terms of the kinetics Ln(C/C$_o$) versus culture time. Data points are averages, and the vertical bars are standard deviations (SD) for samples from duplicate cultures. When the SD bar is absent, the SD is smaller than the point.

media were their salinity (ranging from 19.7‰ to 38‰) and their NH_4^+-N levels (ranging from 2.3 to 7.0 mg L^{-1}), it is evident that *A. carterae* demonstrated a broad tolerance to changes in salinity and [NH_4^+-N]. These observations are in line with other results reported for *Amphidinium carterae*; for example, maximum growth rates at salinities ranging between 15 ‰ and 35‰ were documented for the strain *A. carterae* Hulburt; however, there was no growth below 15‰ (McLachlan, 1961). Therefore, hyposaline stress can be confirmed as the cause of the premature cell death observed in the Z75 culture (Fig. 2G), carried out at a salinity of 10.6 ‰, compared to the ZS75 culture, which was conducted at 38‰. Obviously, the hyposaline stress of Z100 (at 1.4 ‰ salinity) had to be more detrimental than Z75, but the NH_4^+-N-related toxicity also became evident when compared to the ZS100 culture (at 38 ‰ salinity). Z100 and ZS100 were carried out at an initial NH_4^+-N concentration of 9.3 mg L^{-1}. This value is above the 6.3 mg L^{-1} (equivalent to 441 μM) that was established as the approximate NH_4^+-N concentration threshold for *A. carterae* Dn241EHU (Molina-Miras et al., 2020a); in contrast, it is very close to the 7 mg L^{-1} corresponding to Z75 and ZS75.

With regard to P_{bmax}, there was a statistically significant difference between the means of the six culture media (F-ratio = 6.07, p = 0.0242). As can be seen in Fig. 3B, the multiple range tests revealed seven pairs of means with statistically significant differences. Conclusions similar to

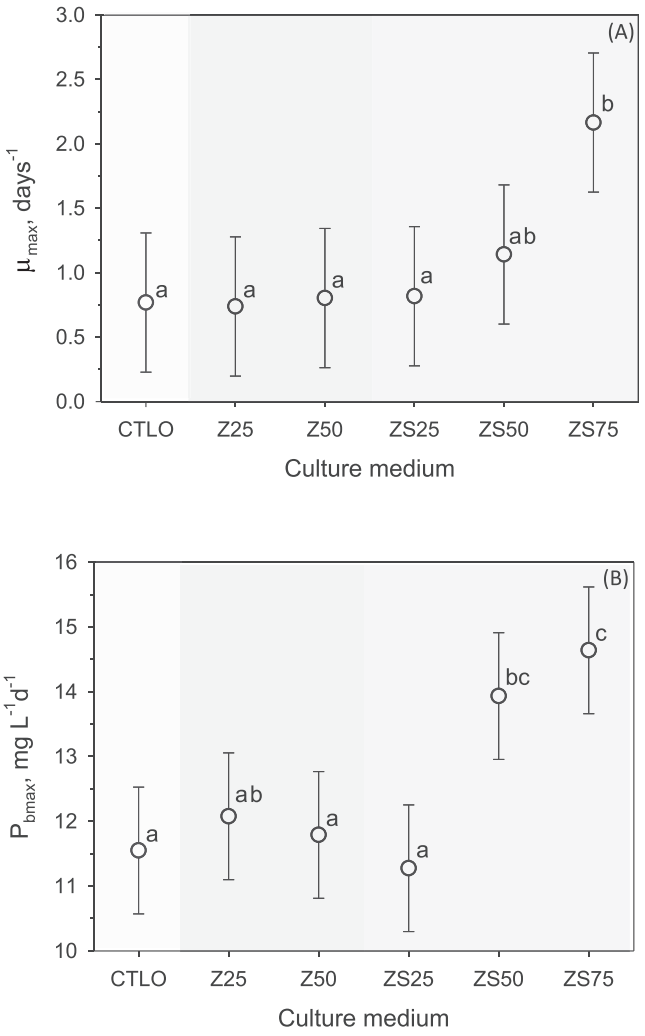

Fig. 3. Evaluation of the effect of the culture medium on (**A**) the maximum specific growth rate and (**B**) the biomass productivity. Values denoted by a different lowercase at each point differ significantly at $p < 0.05$ in the one-way ANOVA. Bars around points represent 95% confidence intervals based on Fisher's least significant difference (LSD) procedure. Overlapping bars indicate no significant difference.

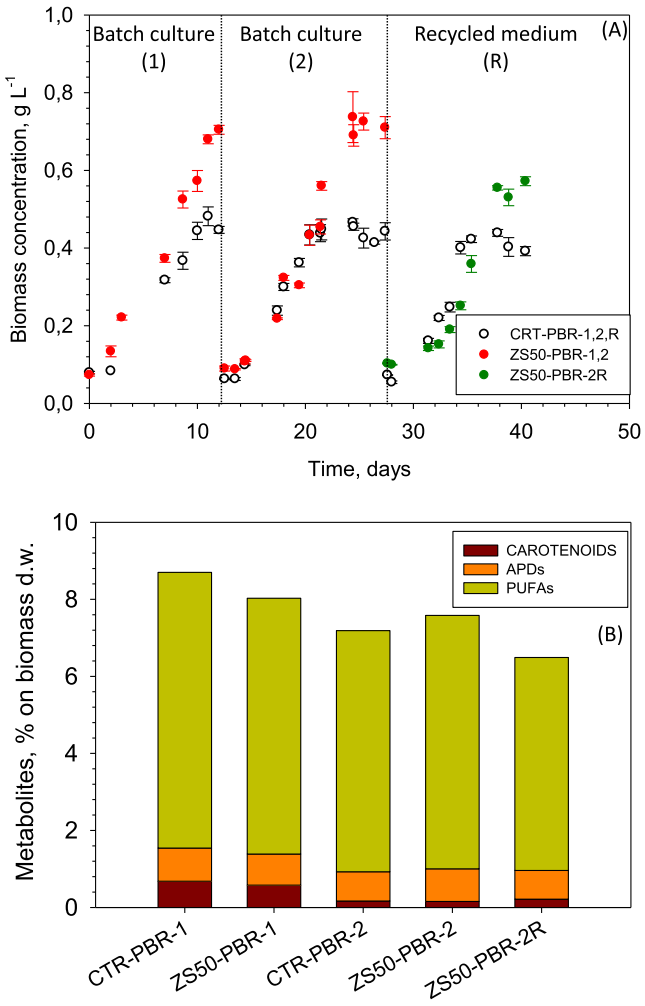

Fig. 4. Effect of the ZS50 medium relative to the control, used both in the sequential batch cultures (1 and 2) and the recycled medium (R) from batch culture 2, on **(A)** the growth kinetics and **(B)** metabolites of interest (amphidinols [APDs], carotenoids and PUFAs) from *Amphidinium carterae* grown in 10-L bubble-column photobioreactors. Data points are averages, and the vertical bars are standard deviations (SD) for samples from duplicate cultures.

those mentioned above for μ_{max} can also be derived from P_{bmax}, with maximum values for ZS50 and ZS75. Based on the results obtained, the use of secondary-treated urban wastewater for growing *A. carterae* is feasible, as long as its ammonium concentration is below the threshold tolerated by the microalgae. The more this threshold is exceeded, the more intensive the zeolite adsorption process and/or dilution with seawater should be. In any case, the hyposaline stress threshold tolerated by the microalga cannot be exceeded; if this happens, adding brine to the culture medium is a viable solution to increase its salinity.

3.3. Proof of concept in bubble-column photobioreactors

Compared to the control medium (CTRL0), any one of the UWW-based media shown in Fig. 3 could have been proposed for a scale-up study. As an example, the ZS50 medium was selected as it provided the maximum biomass productivity and contained an initial NH_4^+-N concentration (4.6 mg L^{-1}) slightly below the threshold value tolerated by the microalga (6.3 mg L^{-1}). Fig. 4A shows the growth kinetics obtained in the bubble-column PBRs using ZS50. Regarding the maximum photochemical yield of photosystem II (Fv/Fm) (data not shown), there were no statistically significant differences between the control and the ZS50 cultures, with average values of 0.53 ± 0.05 and 0.52 ± 0.04, respectively, this being indicative of healthy cells throughout the culture period. The first two sequential batch cultures provided similar final biomass yields, both for the control and for the ZS50 medium, indicating that the cells were acclimated to the respective culture media, at least from the point of view of growth kinetics. The average biomass yield of the ZS50 medium was about 70% higher than the control. Since the control and ZS50 cultures started from the same initial nitrate, phosphate and ammonium concentrations, the increase in the biomass yield in ZS50 had to be due to other sources of nutrients present in the medium; this is likely due to the presence of dissolved organic matter in the wastewater from which ZS50 was formulated (see Table 1). Although, the dissolved organic matter was not analyzed, it is well-recognized that it is of varied etiology with growth-stimulating effects in microalgae cultures (Li et al., 2019).

The enhanced biomass yield was also significant in terms of the recycling of the exhausted supernatant coming from the second batch (Fig. 4A). Nevertheless, the biomass yield was somewhat lower compared to that achieved in the previous two batch cultures. This observation seems plausible considering that a part of the dissolved organic matter present in the wastewater at the beginning of the second culture may have been consumed by the cells, and therefore was not

available to the cells at the beginning of the third culture. In addition, supernatants from microalgal cultures are sinks for a wide variety of compounds excreted by the cells during their growth, mainly as dissolved organic matter, the nutritional benefit of which for the cells is taxa-dependent (Loftus and Johnson, 2017). In the case of *A. carterae*, it was recently proven that the biomass productivity was increased due to the cells mixotrophically consuming the DOM contained in the recycled media (Molina-Miras et al., 2020b).

With respect to the effect of the ZS50 medium on the production of the most interesting metabolites, Fig. 4B details the APD, carotenoid and PUFA contents in the biomass harvested from all the PBR cultures. There were no noteworthy differences between the control and ZS50 within the same set (i.e., CTR-PBR-1 versus ZS50-PBR-1 and CTR-PBR-2 versus ZS50-PBR-2). When comparing the different culture sets (CTR-PBR-1 and ZS50-PBR-1 versus CTR-PBR-2 and ZS50-PBR-2), a significant decrease ($p < 0.05$; 99% confidence level) was only found in the average carotenoid content (of about 74%) between the first set of batch cultures (0.63 % d.w.) and the second set (0.16 %). Regarding the culture with recycled medium (ZS50-PBR-2R), the most notable decrease was observed in the PUFAs content ($p < 0.05$; 99% confidence level), amounting to a 15% decrease relative to the average of the two previous PBR culture sets (6.7% d.w.). The synthesis of APDs was robustly stable in all the PBR cultures, with an average biomass content of 0.79 ± 0.03 % d.w. This result is related to the metabolism of nitrogen and phosphorus in dinoflagellates. The ammonium, nitrate, and phosphate were completely exhausted by the end of all the cultures (data not shown), indicating a growth pattern strongly controlled by N and P availability in the culture medium. It has been reported that N and P limitation of algal growth in dinoflagellates, which are producers of polyketide-type secondary metabolites such as APDs, stimulates their biosynthesis (Van de Waal et al., 2014).

The above results, obtained from short-term PBR cultures, demonstrate that using UWW to cultivate dinoflagellate microalgae is feasible. However, long-term culture studies are necessary to obtain robust acclimation and adaptation cell responses (Molina-Miras et al., 2018; Molina-Miras et al., 2020b). In addition, it is unlikely that long-term cultures of *A. carterea* will remain unialgal when using UWW because the culture medium leads to the presence of microalgal-bacterial consortia.

3.4. Future prospects: The circular economy

The biological conversion of ammonium into N_2 in urban wastewater treatment plants clashes with the development of a circular economy for the current century. Using both the average energy requirements of the Haber-Bosh process to produce 1 kg of NH_3 and the mean ammonium content in a person's excreta per day as the basis for calculation, the energy lost by not recovering ammonium from the UWW may theoretically amount to 38–48% of the embedded chemical energy available in discharged bodily waste (Cruz et al., 2019). To this, one must add the underestimation of the nitrous oxide (N_2O) emissions during the biological treatment of residual waters: N_2O is a potent greenhouse gas that is predominant among ozone-depleting substances (Delre et al., 2017; Domingo-Félez and Smets, 2019). The low ammonium concentration in domestic wastewater (40–60 mg NH_4^+-N / L) contrasts with that found in other sources (>2 g NH_4^+-N / L in digestates, source separated urine and industrial wastewater streams). While ammonium recovery from the latter may be cost-effective, from the former it is not. Taking into account that the cost of recovering low ammonium concentrations should not exceed the cost of ammonium removal, an adsorption-based process is a feasibly option (Cruz et al., 2019). Zeolites, which are very low cost and potent adsorbents, are strong candidates according to a recent comprehensive literature review containing their most relevant uses and applications in biological waste treatment processes (Montalvo et al., 2020). At the same time, recovering different forms of nitrogen (ammonium, nitrites, and nitrates) and phosphorus that are present in wastewater treatment applications through bioconversion with microalgae has been proven to be technically feasible (Abinandan and Shanthakumar, 2015; Liu et al., 2020; Lu et al., 2019). However, microalgal tolerance to ammonium is species dependent. As a result, different tolerability thresholds and toxicity symptoms have been reported (Salbitani and Carfagna, 2021). The two ammonium-recovery concepts are not exclusive and may be combined in several ways. For example, the integration of zeolite-based adsorption systems with specific liquid–liquid membrane contactors allows the production of high-quality liquid fertilizers (for use in agriculture) that have no transition-metal and non-metal ions, or organic micro pollutants (Sancho et al., 2017). These fertilizers may also be used as nutrient sources in industrial microalgae cultures; the culture media would be prepared based on the ammonium concentration tolerated by the microalga and the ammonium supply strategy of the photobioreactor (fed batch, continuous or discontinuous cultures, etc). Another option that has been addressed in this study might be to decrease the UWW ammonium concentration using zeolite adsorption to achieve levels that are tolerable to the microalgae, and/or to use the ammonia-saturated zeolite to slowly provide ammonia to microalgae cultures, thus preventing the toxic effects of this compound (Lu et al., 2019). When dealing with marine microalgae, adapting to hyposalinity conditions is paramount. However, very few marine microalgae tolerate the low salinity of UWW (1-2‰). Consequently, the culture media would need to be supplemented with salts or seawater. In such a case, the proximity of a sewage plant (with an integrated microalgae culture facility) to a seawater desalination plant would be a distinct advantage when it comes to minimizing costs; the various reasons for this are outlined below. Seawater desalination plants generate large volumes of high-salinity brine, which is of significant environmental concern (Roberts et al., 2010). Thus, the brine may be directly applied to adjust the salinities in the microalgal cultures used to recover UWW ammonium. The supernatants of the cultures would be connected to the desalination process so that freshwater and brine could be added. Obviously, the brine production from a seawater desalination plant serving a small city would far exceed that needed for an industrial microalgae culture facility. Nevertheless, the cost of desalinating the supernatants could be offset in several emerging ways: (i) by the price of the microalgal biomass according to its proposed use (as a fertilizer or biostimulant, or to extract high-added-value metabolites, etc.); (ii) by recovering valuable elements from the brine streams (Cs, Rb, U, Li, B, etc.) (Kumar et al., 2021); and (iii) by producing NaOH and HCl from the brine streams for use in a variety of current large-scale industrial activities (Kumar et al., 2019). Interestingly, NaCl, which is abundant in brines, can also be used to regenerate the zeolites (equimolar amounts of Na^+ are required to displace the adsorbed NH_4^+) (Cruz et al., 2019). In summary, applying the circular economy to UWW treatment sustainability will be a key challenge over the coming decades. Zeolites (a recyclable, abundant and natural adsorbent) and microalgae may together form part of the solution for overcoming the obstacles that this objective currently presents.

4. Conclusions

This research has demonstrated that (i) zeolites can be successfully used for treating ammonium-rich wastewater to obtain streams that are tolerable to microalgae; (ii) a sustainable process using marine species, such as *Amphidinium carterae*, to convert the ammonium contained in zeolite-treated UWW into specialty metabolites (e.g., amphidinols, carotenoids and PUFAs) is technically feasible; and (iii) the process described here will generate significant environmental benefits by using brine streams from seawater desalination plants to adjust the salinity of the culture media, regenerating the zeolites with NaCl, and using the desorbed ammonia as a fertilizer.

CRediT authorship contribution statement

L. López-Rosales: Investigation, Conceptualization, Methodology, Data curation, Visualization, Writing – original draft. **P. López-García:** Investigation, Methodology, Data curation. **M.A. Benyachou:** Investigation, Methodology, Data curation. **A. Molina-Miras:** Investigation, Methodology, Data curation. **J.J. Gallardo-Rodríguez:** Investigation, Methodology, Writing – original draft. **M.C. Cerón-García:** Conceptualization, Methodology, Supervision, Writing – original draft, Funding acquisition. **A. Sánchez Mirón:** Conceptualization, Methodology, Supervision, Writing – original draft, Funding acquisition. **F. García-Camacho:** Conceptualization, Methodology, Formal analysis, Visualization, Supervision, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2022.127490.

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