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Nutrient removal from the centrate of anaerobic digestion of high ammonium industrial wastewater by a semi-continuous culture of *Arthrospira* sp. and *Nostoc* sp. PCC 7413

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Abstract
The feasibility of the semi-continuous cultivation of *Arthrospira* (*Spirulina*) sp. and *Nostoc* sp. PCC 7413, using the centrate from the anaerobic digestion of wastewater from a seafood canning industry as a nutrient source, was assessed. Semi-continuous cultures were carried out in aerated tubular culture units of 250 mL by replacing a 10–20% of the volume with the centrate every 2 days. Growth, ammonium, and nitrate removal were monitored, as well as phycobiliprotein and protein production by *Arthrospira* sp. and released exopolysaccharides production by *Nostoc* sp. PCC 7413. The ammonium removal efficiency by *Arthrospira* sp. was 84.9 ± 1.9% in a culture maintained with an ammonium concentration of 11.3 mM. Lower ammonium concentrations (4.9 mM) could be applied to *Nostoc* sp. PCC 7413, reaching similar relative removal efficiency (84.1%). Taking into account the high ammonium stripping that occurs at the high pH required in *Arthrospira* sp. cultures, results indicate a better nutrient removal by *Nostoc* sp., although *Arthrospira* sp. is more suitable for working at high ammonium concentrations. Since all the ammonium was removed during the first 24 h for both species, much lower residence time could be supported by the cultures. High concentrations of protein (2.5 ± 0.2 mg mL^{-1}), phycobiliprotein (allophycocyanin, 84.3 ± 10.2 mg g^{-1}; phycocyanin, 73.7 ± 7.7 mg g^{-1}) were obtained by *Arthrospira* sp. cultures. A high concentration of released exopolysaccharides (962.7 ± 26.7 mg L^{-1}) was also obtained for *Nostoc* sp. PCC 7413 when cultured in the anaerobic centrate. The results indicate the possibility of the use of filamentous cyanobacteria for the treatment of industrial effluents rich in ammonium, with different possibilities of valorization of the biomass produced.

Keywords Wastewater treatment · Anaerobic centrate · Cyanobacteria · Ammonia removal efficiency

Introduction

Microalgal and cyanobacterial biomass is used in highly relevant applications related to human and animal nutrition, and it is also used as raw material for the production of chemicals, biofertilizers, and biofuels (Spolaore et al. 2006). Despite the multiple applications of biomass from microalgae and cyanobacteria, the global production of this type of biomass is less than 20 kt-$year^{-1}$ (Tredici et al. 2016). Microalgae and cyanobacteria biomass production involves vast quantities of nutrients, since based on the elemental composition of microalgae and cyanobacteria, 1.8 kg of CO_2, 0.1 kg of nitrogen, and 0.02 kg of phosphorus are needed to produce 1 kg of biomass (Romero-Villegas et al. 2017). It is possible to use flue gas to supplement CO_2 for algae cultivation; however, fertilizers are usually used for the supply of nitrogen and phosphorus, with imposes an essential limitation on production capacity, reducing the sustainability of biomass production (Collet et al. 2014). The use of fertilizers increases the production cost by around 2.0 € kg^{-1} because it represents up to 54% of the raw material cost (Norsker et al. 2011; Acién et al. 2013). The use of wastewater constitutes a feasible alternative to the use of fertilizers in the production of microalgae and cyanobacteria biomass (Cepoi et al. 2016; Cho et al. 2016). Many types of wastewater are rich in nutrients (carbon, nitrogen, phosphorus, potassium) that are essential to produce these phototrophic microorganisms. Therefore, wastewaters

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can be used for replacing the culture media prepared with fertilizers to produce biomass, which increases the sustainability of the process (Nayak et al. 2016). Moreover, the nutrients present in the wastewater can contaminate various bodies of water (Zinicovscaia 2016) if not adequately removed. Therefore, concurrently with the production of microalgae and cyanobacteria biomass using wastewater for biomass production, the wastewater is treated through the removal of the nutrients, mainly nitrogen and phosphorus (Markou and Georgakakis 2011).

The use of microalgae and cyanobacteria for the secondary treatment of different effluents has been extensively explored mainly in systems such as high-rate algal ponds (HRAP). The use of HRAP in the treatment of domestic wastewater presents economic and environmental advantages, due to the removal of nitrogen and phosphorus, nutrients present in large quantities in this type of waste (Park et al. 2011). Park and Craggs (2010) investigated the influence of the addition of CO_2 on the performance of domestic wastewater treatment and algal production in two pilot-scale HRAPs; with the addition of CO_2, achieving average algal productivity of 16.7 g m^2 d^{-1}, with a maximum algal productivity of 24.7 g m^2 day^{-1}. The effect of the hydraulic retention time (HRT) on the removal efficiency of 26 organic microcontaminants from urban wastewater was investigated in two HRAPs (Matamoros et al. 2015), demonstrating that biodegradation and photodegradation are the essential ways of removing these types of compounds, while volatilization and sorption occurred only in hydrophobic compounds. The cyanobacterium *Leptolyngbya* sp. ISTCY101 has been evaluated for the production of biomass with untreated urban wastewater (1.6 mM NH_4^+), reaching maximum values of biomass productivity of 2.93 ± 0.03 g m^{-2} day^{-1} (Singh and Thakur 2015). Protein production with untreated swine wastewater (4.6 mM NH_4^+) has been demonstrated with *Phormidium* sp., reaching 62% of protein content (Cañizares-Villanueva et al. 1995).

The application of microalgae and cyanobacteria for the treatment of nitrogen-rich wastewater from the aquaculture industry is of particular interest, in the framework of integrated multitrophic aquaculture (IMTA) schemes (Milhazes-Cunha and Otero 2017). The nitrogen and ammonium concentrations that are present in aquaculture effluents are in the range 1.6–17.6 mg L^{-1} and 0.5–5.0 mg L^{-1}, respectively, with an N:P ratio of 9.9:16.2 (Chuntapa et al. 2003; Kamilya et al. 2006; Gao et al. 2016; Wuang et al. 2016), presenting significant differences with urban and other industrial wastewater effluents. High rates of nitrogen removal (83.8 ± 14.0%) were obtained when the cyanobacterium *Spirulina platensis* (now *Arthrospira platensis*; Komárek and Anagnostidis 2005) was co-cultivated with the black tiger shrimp (Chuntapa et al. 2003). Wuang et al. (2016) cultivated *S. platensis* in fish farming wastewater, and the obtained biomass was proved to be an effective biofertilizer, achieved a nitrogen removal efficiency of 80%. Growth and nutrient removal rate of *S. platensis* and *Nostoc muscorum* was evaluated in sewage from a fish culture, showing that the best nutrient removal rates were obtained with *S. platensis*, with 92.4, 48.6, 50.4, and 47.8% for NH_4^+, NO_2^-, NO_3^-, and PO_4^{3-}, respectively, but no significant differences were observed between the specific growth rate of both cyanobacterial species (Kamilya et al. 2006).

Anaerobic digestion of wastewater allows the conversion of organic pollutants into a small amount of sludge and a large amount of biogas (CH_4, CO_2). The main advantages are the low cost of operation, small space required, significant biogas production, and low sludge production (Chowdhury et al. 2010). The use of microalgae and cyanobacteria for the tertiary treatment of the liquid phase of the effluent obtained from anaerobic digestors (centrate) in different wastewater treatment systems has also been explored intensively in recent times, mostly to produce biomass as potential raw material for biofuels, with removal efficiencies reaching 95–98% of nitrogen (Franchino et al. 2013; Ledda et al. 2015a, b, 2016). Some of the eukaryotic species that have been grown successfully on the centrate obtained from the anaerobic digestion of animal manure, sludge from domestic wastewater, and agro-waste include *Neochloris oleoabundans* and *Scenedesmus dimorphus* (Abu Hajar 2017), *Chlorella vulgaris* (Zuliani et al. 2016), *Nannochloropsis gaditana* (Ledda et al. 2015a), *Scenedesmus* sp. AMDD (Bjornsson et al. 2013), and a consortium of *Chlorella* and *Scenedesmus* (Raeisossadati et al. 2019). In the case of cyanobacteria, the centrate from anaerobic digestion of animal manure, activated sludge from domestic wastewater, and distillery wastewater have been used as a culture medium for *Phormidium bohneri* (Noüe and Bassères 1989), *Synechocystis* sp. PCC 6803 (Sadik 2015), and *Spirulina* sp. and *Spirulina platensis* (Chaiklahan et al. 2010; Sankaran and Premalatha 2018). *Arthrospira* (*Spirulina*) sp. has been used in the past to remove nitrogen from anaerobic effluents from digested pig waste (Olguín et al. 2001, 2003; Patnaik et al. 2001).

The high ammonium concentrations reached in the centrate from the seafood/fish canning industry, up to 868 mM or more (Chowdhury et al. 2010; Yenigün and Demirel 2013), together with the vast quantities of organic matter (soluble, colloidal and particulate), phosphorus, sulfur, metals, and solids that are present in this type of effluents, make this type of wastewater suitable for supporting microalgae and cyanobacterial growth (Contreras et al. 2000). Nevertheless, this type of ammonium-rich centrate has not been evaluated for the cultivation of microalgae and cyanobacteria. Therefore, this work aimed to assess the semi-continuous cultivation of filamentous cyanobacteria using the liquid phase of the effluent (centrate) from the anaerobic digestion of a seafood-processing factory as the nutrient source. Filamentous cyanobacteria present the additional advantage of allowing

simple and low-cost harvesting since they can be easily flocculated or sieved (Chen et al. 2014); while producing biomass with a high content of protein (Habib et al. 2008), and other important secondary compounds such as polysaccharides (Otero and Vincenzini 2003) and phycobiliprotein can be obtained simultaneously.

Materials and methods

Anaerobic centrate characteristics

In this research, the centrate utilized was obtained from a 1000 m^3 anaerobic mesophilic digester located in a plant of seafood/fish processing and canning on the NW of Spain (JB Ingenieros, Vigo, Spain). The digester was operated at a flow between 15 and 30 m^3 day^{-1} (average of 25 m^3 day^{-1}), with a regular average biogas production of 600 m^3 d^{-1}. The feed was mainly composed of sewage sludge and cooking water from the fish canning industry, and occasionally dairy industry and slaughterhouse sludges. After the anaerobic digestion, a solid/liquid separation is performed. The liquid stream is sent to a sewage treatment plant and the solid is used as fertilizer. Samples of the centrate of the anaerobic digestion were taken at the outlet of the solid/liquid separation, presenting the following characteristics: NH_4^+ 49.33–113.22 mM, salinity 11–15‰, PO_4-P 0.04–0.17 mM, COD 2090 mg L^{-1}, pH 7.7, conductivity 12.7 mS cm^{-1}, total solids 4450 mg L^{-1}, and volatile solids 2150 mg L^{-1}.

Microorganisms and culture conditions

The cyanobacterium *Nostoc* sp. PCC 7413 was obtained from the culture collection of the Pasteur Institute (https://webext.pasteur.fr/cyanobacteria/). The halotolerant strain of *Arthrospira* sp. was obtained from the culture collection of Microbiology and Parasitology Department of the Universidade de Santiago de Compostela (Spain). Cultures were acclimated to the high concentrations of ammonium by the gradual addition of increasing concentrations of anaerobic centrate for several months, reaching a maximum concentration of ammonium of 6.6 mM and 14.3 mM for *Nostoc* sp. PCC 7413 and *Arthrospira* sp., respectively. During this adaptation period, various volumetric renewal rates and renewal periods were evaluated to establish the renewal rate (RR) and renewal periodicity (RP) that allowed to obtaining better growth and better ammonia removal efficiency (RE). For *Nostoc* sp. PCC 7413, RR 10–20%, and RP of 24–48–72 h were evaluated, while for *Arthrospira* sp., the ranges evaluated were RR 10–15–20% and RP of 24–48 h. Acclimated cultures from log-phase were centrifuged to remove the culture medium and resuspended in fresh $BG11_0$ medium (Allen 1968) for *Nostoc* sp. PCC 7413, and diluted seawater (salinity 20‰), supplemented with $NaHCO_3$ 47.6 mM for *Arthrospira* sp. in order to obtain an initial pH value of 10.0, both of them supplemented with 10% (v:v) of anaerobic centrate. The control cultures were performed with standard BG11 medium (17.7 mM $NaNO_3$, N:P 74) (Allen 1968) for *Nostoc* sp. PCC 7413 and standard Zarrouk medium (29.4 mM $NaNO_3$, N:P 10.1) (Zarrouk 1966) prepared in diluted seawater (salinity 20‰) for *Arthrospira* sp. Cultures were initiated with a high concentration of chlorophyll-*a* (25 ± 0.5 mg L^{-1}), in order to avoid the inhibitory effect of ammonium on growth (Markou et al. 2014).

Cultures were carried out in triplicate in 250 mL culture units with a diameter of 4.6 cm at 30 °C in a thermostatic bath under a circadian lighting regime 12 h light/12 h dark with an irradiance of 168 μmol photons m^{-2} s^{-1}. The bioreactors were continuously aerated with air filtered with 0.2 um cellulose acetate filters (Minisart), supplemented with pulses of CO_2 in order to maintain the pH at 7.8 ± 0.2 for *Nostoc* sp. PCC 7413 and at 9.9 ± 0.3 for *Arthrospira* sp. during the light cycle. Once the early stationary phase was achieved, cultures of *Nostoc* sp. PCC 7413 were maintained in semi-continuous mode by applying a renewal rate of 10% of the volume of the culture with the anaerobic digestate (NH_4^+ 49.33 mM) every other day for 16 days, reaching 90% replacement with the anaerobic digestate on the day 16. Cultures of *Arthrospira* sp. were maintained with a renewal rate of 10% every other day for 8 days, but in this case, the centrate presented a higher ammonium concentration (NH_4^+ 113.22 mM). Due to the decrease in ammonia assimilation observed in the cultures maintained with this high-ammonium centrate, a renewal rate of 20% with a centrate of lower ammonia concentration (49.33 mM) was applied from day ten. It was maintained for 14 additional days, reaching 100% replacement with the anaerobic centrate by day 14. Evaporation (less than 6% of the culture volume) was corrected daily with sterilized distilled water before proceeding to the harvesting and renewal of the culture medium.

Analysis of ammonia stripping under culture conditions

In order to evaluate the loss of ammonia due to stripping, a test in which the decrease of the NH_4^+/NH_3 concentration in uninoculated cultures with an initial concentration of 11.33 mM was performed, maintaining the same conditions of culture medium, pH, aeration and temperature that in the inoculated cultures. Ammonium concentration was measured daily. The non-inoculated cultures were carried out in triplicate under the culture conditions described above.

Analytical methods

Samples (5 mL) were taken daily and centrifuged at 17100 × *g* for 15 min at 4 °C. For the measurement of chlorophyll-*a*, a

modification of the Talling and Driver (1961) method was applied; in brief, the pellet was resuspended in methanol 90% (v:v) and sonicated at 20 kHz for 2 min followed by 1 h extraction at 4 °C. Cell debris was removed by centrifugation, and chlorophyll-*a* absorption was measured spectrophotometrically. Ammonium and nitrate were analyzed in the culture media after biomass removal, with an adaptation of the Nessler method (Krug et al. 1979) and an adaptation of the cadmium reduction method (APHA AWWA WEF 2017), respectively, with a Hanna Instruments analyzer (Hanna Instruments Inc., USA). For *Nostoc* sp. PCC 7413, the released carbohydrates in the culture medium were quantified spectrophotometrically in the supernatant of cultures by the phenol-sulfuric acid method using glucose as standard (Dubois et al. 1956). For *Arthrospira* sp. cultures, protein and phycobiliprotein were also analyzed in the pellets on day 21 (cultures maintained with a renewal rate of 20%). Protein was determined by the Lowry method (Lowry et al. 1951) modified by Herbert et al. (1971), using bovine serum albumin as standard, and phycobiliproteins were measured according to the Bennett and Bogorad method (1973) after resuspending the pellet in 6.0 mL of saline buffer (0.01 M Na_3PO_5–0.15 M NaCl, pH 7.0). Dry weight was measured by filtering 5 mL of biomass and washing with 5 mL HCO_2NH_4 0.5 M three times (Zhu and Lee 1997).

Results

Growth of *Arthrospira sp.* and *Nostoc sp.* PCC 7413 in semi-continuous cultures with anaerobic centrate

Cultures were allowed to grow for 1–2 days in the standard culture media supplemented with 10% centrate, $BG11_0$ for *Nostoc* sp. PCC 7413, and diluted seawater (salinity 20‰), supplemented with $NaHCO_3$ 47.6 mM for *Arthrospira* sp., before starting the semi-continuous operation by renewing 10% of the volume every 2 days with standard culture media or with anaerobic centrate. For *Arthrospira* sp., the control culture maintained in saline Zarrouk medium reached a chlorophyll-*a* concentration of 28.3 ± 0.2 mg L^{-1} at the beginning of the semi-continuous regime. This value was slightly higher than the value obtained in the cultures maintained with 10% centrate (27.6 ± 0.5 mg L^{-1} Fig. 1a). The application of a renewal rate of 10% every 48 h did not produce any change in the chlorophyll-*a* concentration of the control, which recovered the initial concentration after the first 24 h of each dilution cycle, with no further increase in chlorophyll-*a* concentration in the next 24 h of each 48-h renewal cycle, indicating that the cultures were probably nutrient-limited. In the cultures renewed with the anaerobic centrate, the chlorophyll-*a* concentration increased continuously during the first renewal cycles, stabilizing at 28.1 ± 0.4 mg L^{-1} (Fig. 1a), with no significant differences with the cultures maintained with the standard culture medium (Tukey test $P \leq 0.05$). The increase of the renewal rate on day ten from 10 to 20% did not produce any change in the steady-state chlorophyll-*a* concentration either in the control cultures or in the cultures maintained with a centrate, even though the new centrate used presented a lower ammonia concentration (49.33 mM against 113.22 mM centrate used for the dilution 10%). The fact that no growth was recorded during the second 24 h of each renewal cycle regardless of the renewal rate applied excluded the existence of light limitation in the cultures. A gradual increase in the concentration of remaining ammonium in the culture medium was observed between days 6 and 10 (Fig. 1b), indicating that nitrogen was not a limiting nutrient and probably another nutrient, supposedly P could have been limiting the growth of these cultures. No symptoms of physiological stress were observed, as the cells were intact during the whole culture period.

Likewise, for *Nostoc* sp. PCC 7413, no significant differences in chlorophyll-*a* concentration were observed between the cultures maintained with the centrate (final NH_4^+ concentration 4.9 mM) and the cultures maintained with the standard culture medium (Tukey test $P \leq 0.05$) when a renewal rate of 10% was applied every 2 days (Fig. 1c). The values of chlorophyll-*a* obtained in steady state, 28.7 ± 0.4 mg L^{-1}, were equal to those obtained for *Arthrospira* sp. As for *Arthrospira* sp., the values of chlorophyll-*a* on the second day after the renewal were the same as those on the first day, suggesting that the renewal could have been performed daily.

Ammonia stripping

The daily decrease of the NH_4^+/NH_3 concentration caused by the culture conditions (aeration, pH, temperature, culture media), a process known as stripping, was analyzed in uninoculated cultures with the culture medium supplemented with 10% of high ammonium centrate (final ammonium concentration 11.4 mM). Stripping was more pronounced in the Zarrouk medium + anaerobic centrate (pH 9.9), reaching a 28.4% (3.2 ± 0.2 mM) during the first 48 h (Fig. 2). On the contrary, and due to the lower pH of the $BG11_0$ medium, the stripping was much lower, representing a 10.7% (1.2 ± 0.1 mM) of loss (Fig. 2). No increase in nitrate concentration was observed in any condition, indicating that no significant nitrification activity was present.

Ammonium removal

In the semi-continuous culture of *Arthrospira* sp. with centrate, the concentration of the ammonium (NH_4^+/NH_3) was reduced by 80.5 ± 2.8%, from 12.9 mM to values of 2–3 mM after each 48 h renewal cycle during the first four cycles (Fig. 1b). A tendency to decrease the assimilation of

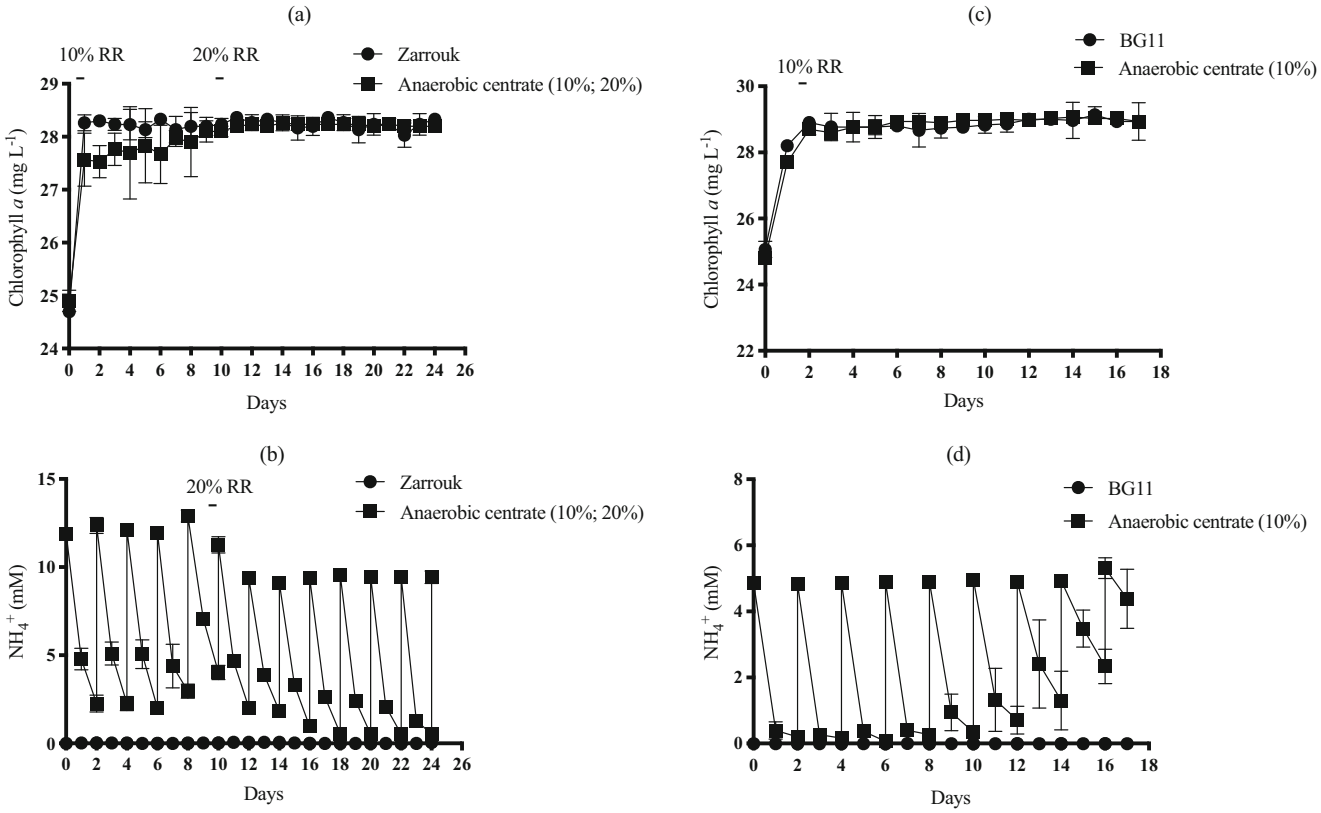

Fig. 1 Growth, measured as chlorophyll-*a* concentration, of (a) *Arthrospira* sp. and (c) *Nostoc* sp. PCC 7413, and changes in NH_4^+ concentration in *Arthrospira* sp. (b) and *Nostoc* sp. PCC 7413 (d). For *Arthrospira* sp. (a and b) arrows indicate the beginning of the semi-continuous operation with a renewal rate 10% (days 1–10, ammonium concentration in the centrate 113.22 mM) and 20% (days 10–24, ammonium concentration in the centrate 49.33 mM). Values are average ± SD ($n = 3$)

ammonium was observed in the last two cycles of 10% renewal (days 8–10), which was not reflected in changes in the concentration of chlorophyll-*a* (Fig. 1a). When a centrate of lower ammonium concentration (49.3 mM NH_4^+) was used, and the renewal rate was increased to 20%, achieving a final concentration of 9.86 mM NH_4^+ after each renewal cycle, ammonium assimilation of 89.8 ± 1.0% was observed (days 12–24), with values of NH_4^+/NH_3 of 0.5–1.0 mM at the end of the 48 h of renewal cycles (Fig. 1b). Most of the ammonium was assimilated during the first 24 h since the NH_4^+/NH_3 removal of the same cultures after 24 h was 64.7 ± 11.3%. Therefore, most of the ammonium decrease during the next 24 h of each renewal cycle can be mostly attributed to stripping. No nitrate concentration was detected in the cultures of both cyanobacteria maintained with anaerobic centrate.

In the cultures of *Nostoc* sp. PCC 7413 maintained with centrate, almost 100% of the ammonium was incorporated at the end of the 2-day renewal cycles, reducing the ammonium concentration from 4.9 to 0.4 mM in the first 24 h, during the first four cycles of renewal (Fig. 1d). Subsequently, the remaining levels of ammonium gradually increased from day 10 to 16, until reaching a concentration of 3.3 mM at the

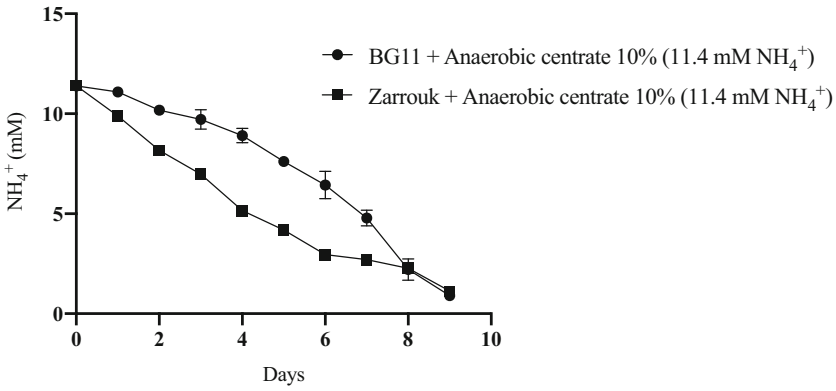

Fig. 2 Ammonium stripping in un-inoculated cultures. Values are average ± SD ($n = 3$)

end of the 9th cycle of renewal, when 90% of the culture medium had been renewed with centrate (Fig. 1d). When observed under the microscope, these cultures of *Nostoc* sp. PCC 7413 showed signs of physiological stress, such as the appearance of disintegrated filaments, which were not observed in the control cultures maintained with the standard culture medium. The deficient concentrations of P in the centrate that resulted in an N:P ratio of 132.5, much higher than the N:P ratios of the culture media (10.1 in Zarrouk and 76.7 BG11), could have caused phosphorus limitation of the cultures, explaining the decreasing trend of ammonium assimilation, which may be dependent of the P provided at the beginning of the cultures.

The percentage assimilation of nitrate in the *Nostoc* PCC 7413 control cultures with BG11 medium (Allen 1968), prepared with fresh water with a nitrate concentration of 17.7 mM, presented average daily incorporation of 9.6 ± 5.0% for *Nostoc* sp. PCC 7413, while the percentage of assimilation of nitrate in the *Arthrospira* sp. control cultures with saline Zarrouk medium (Nitrate concentration 29.4 mM, Zarrouk 1966) prepared with diluted seawater (20‰) was slightly higher 13.1 ± 6.7%. Therefore, none of these cultures were nitrogen-limited.

Production of released exopolysaccharides by Nostoc sp. PCC 7413

Despite the renewal of the cultures every 48 h, the concentration of exopolysaccharides increased continuously in both, the control cultures and the cultures maintained with centrate, with a significantly higher concentration of released exopolysaccharides in the culture maintained with the standard culture media in comparison with the culture maintained with the anaerobic centrate (Fig. 3) (Tukey test $P \leq 0.05$). The concentration of released exopolysaccharides in the cultures maintained with centrate was 962.7 ± 26.7 mg L^{-1} (Fig. 2). This value is 25.0% lower compared with the culture with standard BG11 medium (1282.7 ± 8.4 mg L^{-1}).

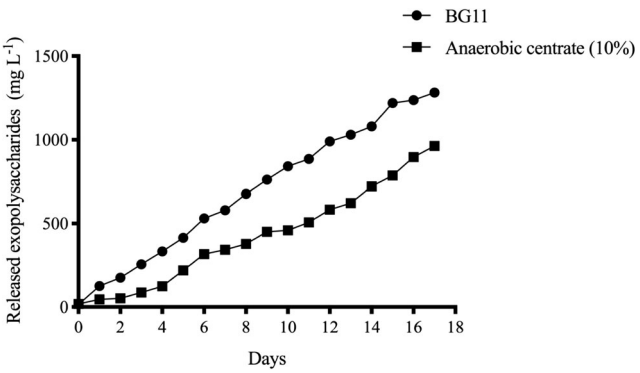

Fig. 3 Concentration of released exopolysaccharides in semi-continuous cultures of *Nostoc* sp. PCC 7413. Values are average ± SD ($n = 3$)

Dry weight, protein concentration, and phycobiliprotein by a semi-continuous culture of *Arthrospira sp.*

Notwithstanding no differences were found in chlorophyll-*a* concentration between control cultures and cultures maintained with centrate (Fig. 1a), the values of dry biomass obtained were significantly different (Tukey test $P \leq 0.05$). The steady-state biomass concentration of the cultures maintained with centrate with a renewal rate of 20% every 2 days achieved a mean value of 5.0 ± 0.1 mg mL^{-1}, whereas in the standard culture medium a mean value of 4.1 ± 0.2 mg mL^{-1} was obtained in the harvested cultures (Table 1) (Tukey test $P \leq 0.05$). This value corresponds to a 22% increase in productivity measured as dry biomass in the culture supplemented with anaerobic centrate and using ammonium as a combined nitrogen source in comparison to culture maintained in saline Zarrouk medium, despite the much higher nitrogen availability in the latter.

The protein concentration was also significantly higher in the culture maintained with centrate that achieved a protein concentration of 2.5 ± 0.2 mg mL^{-1}, corresponding to 49.0% of the dry biomass. In contrast, only 1.7 ± 0.1 mg mL^{-1} of protein were achieved in the culture maintained with the standard culture medium (Turkey's test $P \leq 0.05$) (Table 1).

The phycobiliprotein pigments: allophycocyanin (APC) and phycocyanin (PC), presented lower concentration (mg g^{-1} biomass) in the cultures maintained with centrate compared to cultures with the standard culture medium (Table 1); however, this difference is not statistically significant (Turkey's test $P \leq 0.05$).

Discussion

The results obtained demonstrated the feasibility of maintaining a stable semi-continuous culture of the cyanobacteria *Arthrospira* (*Spirulina*) sp. and *Nostoc* sp. PCC 7413 using the centrate from the anaerobic digestion of a seafood/fish canning plant as a source of nutrients, achieving at the same time a high rate of ammonium removal and constituting an

Table 1 Dry biomass, protein and phycobiliprotein (Allophycocyanin and Phycocyanin) of *Arthrospira* sp. in semi-continuous cultures

Growth parameters	Zarrouk	Anaerobic centrate
Dry weight	4.1 ± 0.2 mg mL^{-1}	5.0 ± 0.1 mg mL^{-1}
Protein	1.7 ± 0.1 mg mL^{-1}	2.5 ± 0.2 mg mL^{-1}
Phycobiliprotein:		
Allophycocyanin	101.1 ± 9.4 mg g^{-1}	84.3 ± 10.2 mg g^{-1}
Phycocyanin	87.1 ± 7.2 mg g^{-1}	73.7 ± 7.7 mg g^{-1}

Values are average ± SD ($n = 3$)

interesting alternative for tertiary wastewater treatment, helping to avoid the environmental impact of these nutrients on the bodies of water. In addition to having the ability to use ammonium (NH_4^+/NH_3) as a combined nitrogen source for its metabolism, a compound that at certain concentrations is toxic, these cyanobacteria species can also be used in animal feed (Habib et al. 2008), as fertilizer and other high added-value applications such as the production of polysaccharides (Arias et al. 2020) or pigments. Another advantage of filamentous cyanobacteria is the lower cost of harvesting the biomass. The use of filamentous species constitutes a way to solve the problem of harvest associated with the species of unicellular microalgae traditionally used in wastewater treatment systems (Gerardo et al. 2015; Liu et al. 2020). Bioproducts from microalgae are promising for the future; however, the current microalgal cultivation cost is too high to allow many commercial applications. Since nutrient use may account for half of the cost of microalgal cultivation (Norsker et al. 2011; Acién et al. 2013; Craggs et al. 2013), wastewater and other liquid streams rich in nutrients could represent suitable substrates to sustain algal biomass production with an additional positive environmental impact (Hammed et al. 2016).

The results obtained in this work with the anaerobic centrate of a seafood/fish canning industry indicate the viability of the use of *Arthrospira* sp. and *Nostoc* sp. PCC 7413 in the bioremediation of this type of effluents, with the potential of becoming an alternative for the standard tertiary treatment due to its high capacity for nutrient removal. It was possible to obtain removal efficiencies of NH_4^+/NH_3 in the range 73.4–56.5% in cultures with anaerobic centrate, at a final ammonium concentration of 4.9, and 11.9 mM for PCC 7413 and *Arthrospira* sp., respectively. Although the retention times used in this preliminary experiment are high, with renewals applied every 48 h, the data indicate that much lower retention times could be applied, since most of the ammonium was retrieved in the first 24 h. For the cyanobacteria, *Phormidium bohneri* cultured with anaerobic centrate from swine manure, an *RR* of 50% and an *RT* of 96 h were used, with a final ammonium concentration of 1.8 mM (Noüe and Bassères 1989). Wang et al. (2010) evaluated the semi-continuous cultivation of *Chlorella vulgaris* in anaerobic centrate of cattle manure with a final ammonium concentration of 3.9 mM. They worked with *RRs* of 5, 10, and 20% with an *RT* of 24 h; the results showed that with an *RR* of 20%, the microalgal biomass declined, while with an *RR* of 10%, the growth was stable but the ammonia removal efficiency (*RE*) was insufficient (58.3 ± 0.01). An *RR* of 5% every 24 h was selected as optimal since *RE* increased significantly (100.0%). In cultures of the cyanobacterium *Synechocystis* sp. PCC 6830 and the microalga *Nannochloropsis salina* with an anaerobic centrate of municipal wastewater (final ammonium concentration of 7.6 mM), two *RR* (25–50%) and various *RT* (24 to 288 h) were evaluated. The highest biomass productivity was achieved with an *RR* of 50%, and an *RT* of 48 h; however, the removal efficiency (*RE*) of N and P decreases significantly with this low *RT*, the best *RE* of both nutrients was achieved with an *RR* of 50% and an *RT* of 144 h (89.0 ± 1.7 and 82.8 ± 1.8, respectively) (Cai et al. 2013a, b). An *RR* of 10% and an *RT* of 24 h were applied to a consortium of *Chlorella* sp. and *Stigeoclonium* sp. and the cyanobacterium cf. *Oscillatoria* maintained with an anaerobic centrate of the final NH_4^+ concentration of 1.8 mM (Arias et al. 2017). We must emphasize that the significantly lower ammonium concentrations are used in the studies mentioned above that the ones used in our study.

Also, the values of chlorophyll-*a*, in *Arthrospira* sp., and *Nostoc* sp. PCC 7413 on the second day after renewal were the same as those on the first day, suggesting the presence of either-or nutrients limitation. Although a slower but significant growth of the cultures during the second 24 h of each renewal cycle would have been expected in the case of light-limited cultures, the light limitation cannot be entirely discarded. Therefore, higher levels of irradiance should be evaluated in order to determine their influence on the nutrient removal capacity of the cultures. The values of chlorophyll-*a* concentration obtained were similar in the control cultures and in the cultures maintained with centrate, indicating that the nutrient levels present in the centrate are enough to sustain active growth. Only in the cultures of *Nostoc* sp. PCC 7413, a decrease in nitrogen removal was observed in the last renewal cycles (Fig. 2b), probably related to the observed disintegrated filaments that could be indicative of physiological stress and were not observed in the control cultures maintained with the standard BG11 culture medium. The low P content of the centrate used in this experiment that resulted in high N:P ratios could have caused phosphorus limitation of the cultures, explaining the decreasing trend of ammonium assimilation, which may depend on the P provide at the beginning of the cultures. This limitation was not revealed in *Arthrospira* sp. cultures, probably due to the higher P concentration present initially. Therefore, the concentration of P should be monitored and corrected in this type of industrial effluents to guarantee high nitrogen removal efficiency. However, under certain conditions, the fish processing residues digestate can present high molarities of this nutrient, achieving values as high as 6–10 mM (Guerrero et al. 1999), and therefore not requiring nutritional correction. It would nevertheless be necessary to carry out additional studies to confirm the limiting role of P in these effluents.

Results described by other authors show the feasibility of the culture of microalgae and cyanobacteria in anaerobic centrates. Abu Hajar (2017) cultivated the microalga *Neochloris oleoabundans* in a diluted anaerobic centrate from the anaerobic digestion of animal manure with an ammonium concentration of 7.8 mM obtaining removal efficiencies (RE) of NH_4^+/NH_3 in the range 64–78%; Uggetti et al. (2014) used

the centrate from anaerobic digestion of urban wastewater for the culture of *Scenedesmus* sp. with an ammonium concentration in the range of 2.8–14.4 mM, and demonstrated that high concentrations of ammonium decreased the growth rate from 0.9 to 0.04 day^{-1}. Bjornsson et al. (2013) cultivated the microalgae *Scenedesmus* sp. AMDD with the centrate from the anaerobic co-digestion of algal biomass and swine manure (1.5 mM NH_4^+) with and without CO_2 addition; and obtained a maximum dry weight of 0.27 g L^{-1} without the addition of CO_2, and 0.42 g with its addition, achieving an ammonium RE of 99.6%.

The concentration of chlorophyll-*a* obtained in the cultures maintained with centrate is similar to that reported by other authors with *Nostoc* sp. Otero and Vincenzini (2003), evaluated PCC 7413 in a similar culture system under continuous illumination, obtaining maximal chlorophyll-*a* values of 28.0 ± 0.5 mg L^{-1}. In the case of *Arthrospira* sp., the concentration of chlorophyll-*a* in our cultures was significantly higher than that described by other authors in wastewater, even though we were using diluted seawater in the control cultures. Olguín et al. (1994) cultivated *Arthrospira maxima* in HRAPs at 30 °C, with seawater (salinity 30‰) supplemented with 2% (v:v) of centrate of pig purines with a final ammonium concentration of 1.4 mM, obtaining a maximum concentration of chlorophyll-*a* of 8.1 mg L^{-1}, a value significantly lower than the concentration obtained in this work (28.3 ± 0.2 mg L^{-1}). The lower ammonium concentration and irradiance (70 μmol photons m^{-2} s^{-1}) used in those cultures and the lack of addition of CO_2 may be responsible for the lower growth and removal capacity.

Arthrospira sp. presents a lower ammonium RE due to high stripping (56.5%), compared with *Nostoc* sp. PCC 7413 (73.4%). However, *Arthrospira* sp. ammonia assimilation capacity was significantly higher (1.4 times) than that obtained with *Nostoc* sp. PCC 7413. This characteristic, together with the high percentage of protein (50%) of the dry biomass produced in this type of wastewater, makes it interesting for the production on a larger scale. Furthermore, as reported by other authors (Abeliovich and Azov 1976; Belkin and Boussiba 1991), we confirm the high tolerance of *Arthrospira* sp., being an alkalophilic species, to high ammonium concentrations. However, it should be noted that PCC 7413 presented a high ammonium removal efficiency, and a daily ammonium consumption slightly lower than that of *Arthrospira* sp., presenting the additional advantage of its significant production of released exopolysaccharides with these residual (962.7 ± 26.7 mg L^{-1}). This value of EPS is significantly lower than the values reported for this species in previous studies (1.8 g L^{-1}, Otero and Vincenzini 2003). This can be caused by a higher light availability in the latter, derived from the application of continuous illumination. The lower production of exopolysaccharides in centrate cultures compared with the culture with the standard culture medium may due to the fact the ammonia can deviate the flow of carbon to the synthesis of protein in detriment to that of carbohydrates (Lawrie et al. 1976). Both the assimilation of nitrate and ammonium affect the rate of CO_2 fixation and the carbon flux to a different metabolic fraction (Romero and Lara 1987). Cañizares-Villanueva et al. (1995) obtained a higher protein: carbohydrate ratio (3.9) for the filamentous cyanobacteria *Phormidium* sp. when cultivated in porcine wastewater with 2.3 mM NH_4^+, compared with the control maintained with KNO_3 (1.9), despite the much higher nitrogen availability in the latter.

Very high values of dry weight, 5.0 mg mL^{-1}, and protein concentration (2.5 mg mL^{-1}) were obtained in the cultures of *Arthrospira* sp. grown with the anaerobic centrate, being higher those obtained in the standard culture media and those obtained by other researchers. Bezerra et al. (2007) obtained a maximum dry biomass concentration of 1.9 g L^{-1} in fed-batch cultures of *A. platensis* with an initial concentration of 1.7 mM NH_4Cl. The lower ammonium concentration used in those cultures and the lack of addition of CO_2 may be responsible for the lower dry weight. Results closer to those reported here were obtained by Delrue et al. (2017) that cultivated *A. platensis* in a modified Zarrouk medium with a high inoculum concentration reaching a final dry weight of 3.4 g L^{-1}, a value similar to that obtained here for standard Zarrouk medium, 4.1 g L^{-1}. The high biomass values obtained here may be due to the high effective irradiance available in the tubular systems (diameter 4.6 cm) and strict pH control. In fact, up to 6.0 g L^{-1} was achieved in *Chlorella sorokiniana* cultures in synthetic urine with an irradiance of 990 μmol photons m^{-1} s^{-1} (Tuantet et al. 2014).

The result obtained in this work for the protein fraction of *Arthrospira* sp. cultivated with centrate from the effluent from a fish canning industry is similar to those obtained by other authors with centrate from other types of effluents. In semi-continuous cultures of *Arthrospira* with diluted seawater supplemented with the centrate of pig purines presenting a lower concentration of ammonium (2.2 mM), the protein concentration ranged from 35 and 49%, which are similar to the value obtained with the fish canning centrate (49% w/w) in this research (Olguín et al. 2001). Only in one case with higher protein content values, 71% w/w have been reported in cultures of a mutant strain of *S. maxima* growing at 30 °C with constant illumination (60–70 μmol photons m^{-2} s^{-1}) growing in seawater supplemented with anaerobic centrate from pig waste (2% v/v) (Olguín et al. 1994).

Conclusion

Our results indicate that the filamentous cyanobacteria *Arthrospira* sp. and *Nostoc* sp. PCC 7413 can be produced using centrate from the anaerobic digestion wastewater from a

seafood/fish canning plant as the nutrient source, representing an interesting alternative for the treatment and valorization of nutrient-rich anaerobic digestion effluents. However, additional experiments are required in order to assess the feasibility of the treatment on a large scale.

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