Potential of a local microalgal strain isolated from anaerobic digester effluents for nutrient removal

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Abstract

Anaerobic digestion effluents contain nitrogen and phosphorous which require reduction to accomplish the corresponding discharge legislation. Microalgae can be used as an alternative treatment to reach the required effluent quality. However, only robust and fast growing microalgae species are required in order to ensure stable and efficient nutrient removal under the conditions existing in wastewater treatment plants. Consequently, maintaining a stable microalgae community adapted to this environment becomes a key issue. In this work, a local microalgal strain was isolated from an anaerobic digester effluent (ADE). Microalgal growth was defined as the isolation criteria. The isolated microalgae were identified by molecular techniques as *Chlorella sorokiniana* (strain S12/S13/S16). Nutrient removal capacity from the ADE was assessed for the isolated strain by cultivation on ADE in repeated batch mode. Growth was limited by phosphorus, which reached removal efficiencies close to 100%. Under such conditions, biomass productivity and growth rate were barely enhanced with CO_2-enriched air not compensating the extra cost of CO_2 addition. Finally, in order to close the process scheme, the methane potential of the isolated *C. sorokiniana* was assessed. The methane production capacity was 376 mL CH_4 g^{-1} volatile solids, similar to values reported for other microalgae species. The novelty of this work lies in the isolation of a robust local microalgal strain that ensures a high nutrient removal capacity from ADE. The efficiency and stability of the nutrient removal process might be enhanced by isolation and controlled growth of local, robust, and also fast-growth microalgae species.

Keywords

Anaerobic digestion
Algae
Bioprocesses
Nutrient removal
Wastewater treatment

Introduction

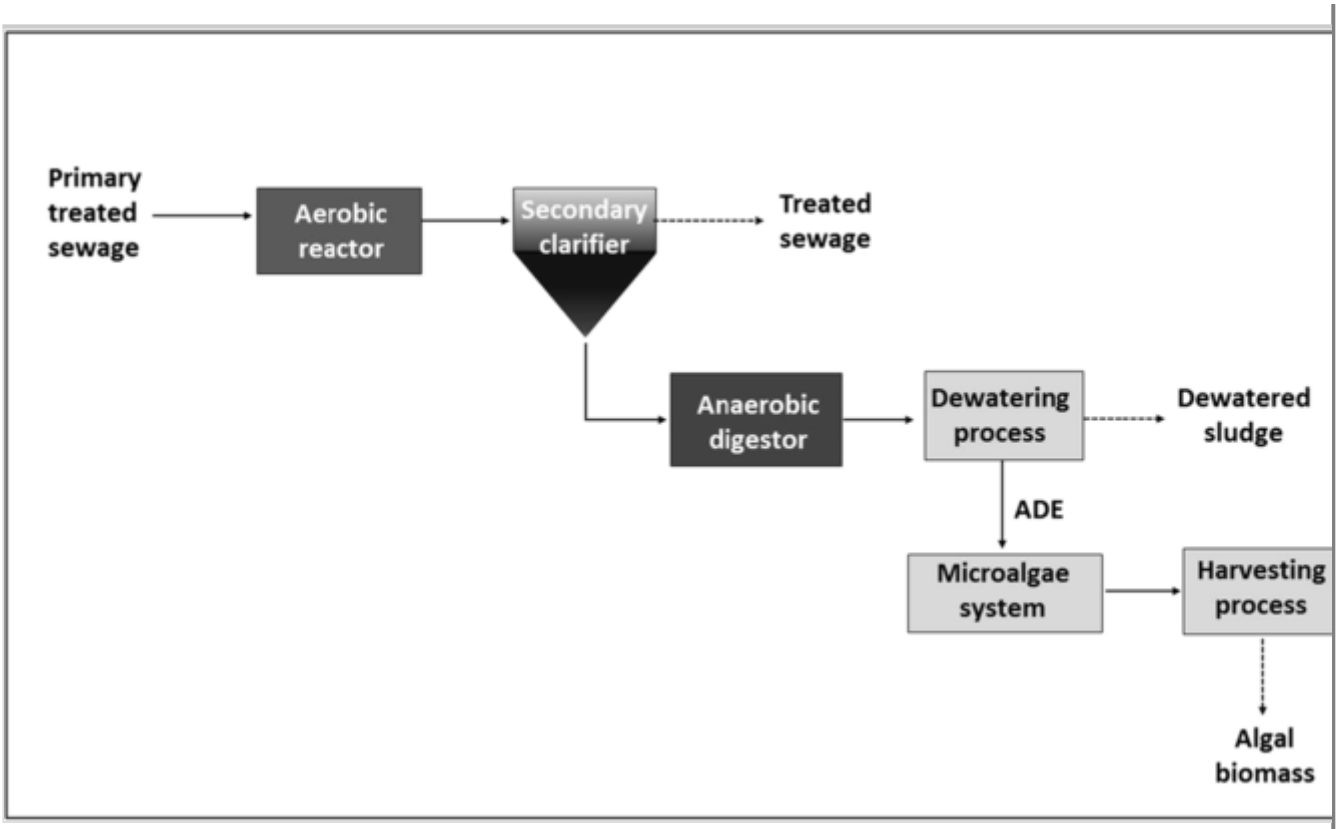
Water pollution is no doubt a major issue for human health in the new century. Some water pollutants including inorganic nitrogen and phosphorus are not

efficiently removed by traditional wastewater treatment processes, resulting in eutrophication of aquatic ecosystems (Dhir 2014). Inorganic nitrogen and phosphorus promote the growth of photosynthetic organisms, such as microalgae, which have the ability to efficiently use such nutrients (Martínez et al. 2000; de Godos et al. 2014; Forján et al. 2015). That capability can be converted in a great advantage if used for nutrient recovery under controlled conditions in wastewater treatment plants. However, only robust and fast growing microalgae species are preferred in order to ensure stable and efficient nutrient recovery processes under the conditions existing in wastewater treatment plants. Consequently, maintaining a stable microalgal community adapted to this environment becomes a key issue.

Conventional wastewater treatment plants are usually composed of a primary settler followed by an aerobic reactor, where sludge is produced through organic matter degradation. This aerobic sludge produced in the aerobic reactor is usually stabilized by anaerobic digestion. The clarified effluent of the aeration step contains a low concentration of nitrogen and phosphorus, but they may still require further reduction to accomplish the corresponding discharge legislation. Moreover, anaerobic digestion effluent (ADE), the liquid fraction obtained after digested sludge dewatering, provides a nutrients source that should also be treated (Fig. 1). Microalgal growth can be used as an alternative treatment to reach the required effluent quality.

Fig. 1

Proposed wastewater treatment system with nutrient recovery from anaerobic digester effluent by microalgae treatment



Suitability of ADE as nutrient source for microalgal growth will mostly depend on the microalga's robustness, on the adaptation to grow in presence of biological competitors, on the algal ability to profit from the interaction with some of these theoretical competitors through specific microalgal-bacterial interactions (Fuentes et al. 2016), and on the algal affinity for the nitrogen and phosphorus sources (Kobayashi et al. 2013; Kwon et al. 2013; de Godos et al. 2014). For the above reasons, the study of microalgae species naturally occurring in the ADE, adapted to assimilate nutrients from such a physical-chemical-biological complex scenario, becomes relevant to determine which microalgae perform the nutrient removal process from ADE better. Other previous studies have evaluated the use of local microalgal isolates for their ability to grow and remove nutrients from different wastewaters such as primary/secondary wastewater effluents and digested swine manure wastewater (Zhou et al. 2012; Bohutskyi et al. 2015). However, the novelty of this work lies in the use of microalgal strains isolated from the ADE itself and which might be naturally adapted to grow and remove nutrients from such effluent.

In a sequential process in which microalgae are grown in ADE effluents, the resulting microalgal biomass will be harvested in order to allow the separation of the final clean effluent and the microalgal biomass. The microalgal biomass will contain most of the nutrients from the ADE effluent and that biomass could be further valorized (i.e., as fertilizer) (Coppens et al. 2016). Another option could be to co-digest the biomass for methane production (Neumann et al. 2015;

Beltrán et al. 2016). The methane productivity of a given microalga species depends on process conditions, microalgae chemical composition, and particularly, on the cell wall digestibility (The reference Afi et al. 1996 should be cited here instead of Retfalvi and Inglesby. Info about the reference Afi has been included in the reference list.

As commented in the next comments, Retfalvi and Inglesby were not in the manuscript and they are suggested to be removed Chen and Oswald 1998; Sialve et al. 2009; Inglesby That reference was not in the manuscript and therefore it is suggested to be deleted from the text and from the reference list et al. 2015; Fermoso et al. 2016; Rétfalvi That reference was not in the manuscript. It is suggested to delete the citation from the text and also from the reference list et al. 2016). In a given time period, a fast-growth microalga species fixes an amount of inorganic carbon which is higher than that fixed by slow-growth species. This results in a high productivity of algal biomass, which means a high amount of readily available carbon for methane production. Consequently, the use of fast growth microalgae species also contributes to increasing methane production rate considering the whole production process chain.

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The overall objective of this work was to assess the efficiency and stability of nutrient removal from ADE when performed by a dominant, specific algal species isolated from the ADE itself. The specific aims of this research were (a) to isolate and identify the fastest-growth microalga species present in the ADE, (b) to evaluate the microalgal growth kinetics in ADE media in batch and feed-batch mode, and (c) to evaluate the potential energy recovery of the obtained microalgal biomass through anaerobic digestion.

Materials and methods

Microalgae isolation and selection

The microalga used in this work was isolated from an anaerobic digestion effluent (ADE), which was taken from a continuously stirred tank reactor for sewage sludge biomethanization (Wastewater Treatment Plant El Copero, Seville, Spain). Samples of ADE were taken in order to inoculate Petri dishes containing Sueoka synthetic medium (Sueoka 1960). Microalgae colonies were isolated from Petri dishes through standard serial plating techniques in 10-mL tubes and grown in culture room for 7 days. Cultures were incubated at 25 °C and illuminated at 150 μmol photon m^{-2} s^{-1} with white fluorescent light. Subsequent growth experiments were performed to evaluate and choose the fastest growing microalga. To do that, each 10 mL tube sample was used to inoculate 100-mL Erlenmeyer flasks under same cultivation conditions. The

algal density of the cultures was measured by optical density at 750 nm after 3-day period. The fastest growing microalga was chosen for identification and subsequent experimental phases.

Microalgae identification

Isolated microalga identification was carried out by extracting and purifying genomic DNA through MoBioUltraClean PowerSoil DNA Isolation kit (MoBio Laboratories Inc., cat no. 12888-100, USA) according to manufacturer procedure. The 18S ribosomal RNA gene was amplified from genomic DNA using the two pairs of primers EUK-1A (5′-CTGGTTGATCCTGCCAG-3′) and EUK-516R (5′-ACCAGACTTGCCCTCC-3′), 18S-C (5′-TGATCCTTCYGCAGGTTCAC-3′) and 18S-D (5′-ACCTGGTTGATCCTGCCAG-3′) (Moreno et al. 2013). The DNA amplification was carried out in a Thermal Cycler AB2720 (Applied Biosystems, Thermo Fisher, USA) with Sapphire Fast PCR Mastermix (cat N° RR350A, Takara Bio Inc., Japan) using the following PCR conditions: an initial denaturation at 95 °C during 30 s followed by 35 cycles of 30 s at 95 °C, 60 s annealing at 67 °C, and 60 s extension at 72 °C, followed by 300 s at 72 °C after the last cycle. The PCR products were purified through Promega DNA Purification Kit (Promega, USA) and sequenced by Macrogen Inc. (South Korea). The sequence was edited and assembled by using ChromasPro 1.5 software and analyzed through CLC Main Workbench (version 6.7.1) and subsequently analyzed using BlastN software against the non-redundant database available in GenBank (www.ncbi.nlm.nih.gov/blast/Blast/cgi) with a cutoff of 1×10^{-5}.

The sequences were aligned and compared with a dataset of green algal sequences from Zhang et al. (2008) and GenBank using ClustalX software (Thompson et al. 1997) from MEGA6. The alignments were realized in MEGA6 (Tamura et al. 2013). The phylogenetic tree was generated with a maximum likelihood method based on the general time reversible model (Nei and Kumar 2000).

The isolated and identified strain can be obtained from the culture collection of Central Research Services (CIDERTA) of the University of Huelva, Huelva, Spain.

Microalgae growth in batch mode

The selected microalga was grown under phototrophic conditions in Sueoka synthetic medium (Sueoka 1960) and in ADE medium. ADE medium was prepared by centrifugation of crude ADE (10 min at 3000×*g*) followed by filtration of the obtained supernatant (0.7 μm pore size, glass microfiber filters)

(MFV-5, Filter-Lab, Filtros Anoia, Spain). Table 1 shows the chemical composition of the employed ADE medium. In order to compare the composition of the ADE medium with other effluents used in previous works with microalgae, related information has been also included in Table 1. As can be seen, the ADE medium used in this study had a similar composition to other reported ADE. The growth of the isolated microalga in a suitable, non-nutrient limited Sueoka medium should allow obtaining the maximal microalgal productivity that might be expected from the microalga cultivated under the experimental conditions of this work. Such maximal productivity should be a suitable reference to compare it with the growth efficiency of the isolated microalga in ADE medium.

Table 1

Chemical composition of the anaerobic digestion effluent (ADE) used in this study compared to other effluents used in similar studies

	Parameters (mg L^{-1})							
ADE origin	**COD**	**Total N**	**N-NH_4^+**	**N-NO_3^-**	**N-NO_2^-**	**Total P**	**PO_4^{3-}**	**Reference**
WWTP	334	–	626	56.7	0.3	–	9.23	This study
WWTP	6900	928	825	84	–	45.72	39.68	Ji et al. (2014)
Pig manure	1042	1220	1196	–	–	75	–	Park et al. (2010)
WWTP	1250	2897	2845	9.30	–	425	–	Sheets et al. (2014)
	Parameters (mg L)							
ADE origin	**COD**	**Total N**	**N-NH**	**N-NO**	**N-NO**	**Total P**	**PO**	**Reference**
Dairy wastewater	920	596	470	–	–	–	29	Zielinski et al. (2018) AQ5

The batch assays were carried out in 1-L Erlenmeyer flasks, incubated at 25 °C and illuminated with white fluorescent lamps (150 μmol photons m^{-2} s^{-1} PAR irradiance measured at the flasks surface). Microalgal biomass concentration was adjusted to 0.1 g L^{-1} at the beginning of the experiment. The Erlenmeyer flasks containing Sueoka medium were supplemented with carbon dioxide by bubbling CO_2 enriched-air (5% *v*/*v*) into the cultures. The Erlenmeyer flasks containing ADE medium were exclusively sparged with air due to the presence of organic

carbon in the ADE. The growth experiments were carried out in triplicate. The described culture conditions were kept constant in all the experiments.

Microalgae growth in feed-batch mode

The isolated microalga was grown in ADE medium under feed-batch conditions in order to study the long-term ability for inorganic nitrogen and phosphorus removal from ADE. Feed-batch cultivation was carried out under the same conditions explained above for the batch mode experiments. Biomass from the batch mode experiments in exponential phase was used as inoculum and microalgal biomass was maintained in the range of 0.35 to 0.55 g L^{-1} by periodical dilution with ADE medium. Microalgae growth in cultures sparged with air was compared to cultures bubbled with CO_2-enriched air (5% *v*/*v*) to evaluate a possible limitation of carbon, instead of nitrogen or phosphorus and, presumably, to obtain the maximal microalgal productivity.

Biomethane potential tests

The microalgal samples used in the BioMethane Potential (BMPs) tests were obtained from the fed-batch mode experiments. Once steady phase in the fed-batch mode experiments was reached; the microalgal biomass was harvested by centrifugation (10 min at 3000 × *g*) and freeze-dried prior to the BMP tests. BMP tests were performed in 250 mL serum bottles with 130 mL of working volume. An initial microalgal biomass concentration of 4 g L^{-1} of volatile solids (VS) was used. The anaerobic inoculum to microalga biomass ratio was 2:1 in VS. The BMP tests were supplemented with sodium bicarbonate (0.5 g L^{-1}) and 0.1 mL of mineral traces containing: 200 mg L^{-1} $FeCl_2 \cdot 4H_2O$, 200 mg L^{-1} $CoCl_2 \cdot 6H_2O$, 500 mg L^{-1} $MnCl_2 \cdot 4H_2O$, 90 mg L^{-1} $AlCl_3 \cdot 6H_2O$, 50 mg L^{-1} H_3BO_3, 50 mg L^{-1} $ZnCl_2$, 38 mg L^{-1} $CuCl_2 \cdot 2H_2O$, 50 mg L^{-1} $NiCl_2 \cdot 6H_2O$, 194 mg L^{-1} $NaSeO_3 \cdot 5H_2O$, and 1000 mg L^{-1} EDTA. Methane production was determined based on NaOH volume displacement. The BMP value was evaluated considering accumulated methane production. Endogenous biogas production from anaerobic biomass was determined by blank assays containing only inoculum. All BMP tests were performed in triplicates.

Analytical methods

Total suspended solids (TSS), volatile solids (VS), and the concentration of N-NH_4^+, N-NO_2, and PO_4^{3-} were determined according to standard methods described by APHA (APHA 2005). N-NO_3^- was determined according to the method described by Cawse (1967). The elemental composition of microalgal biomass (carbon and nitrogen) was determined by using an elemental analyzer

system, LECO CHNS-932 (Leco Corporation, USA). The growth rate and biomass productivity were calculated according to Vaquero et al. (2014).

Results and discussion

Microalga isolation and identification

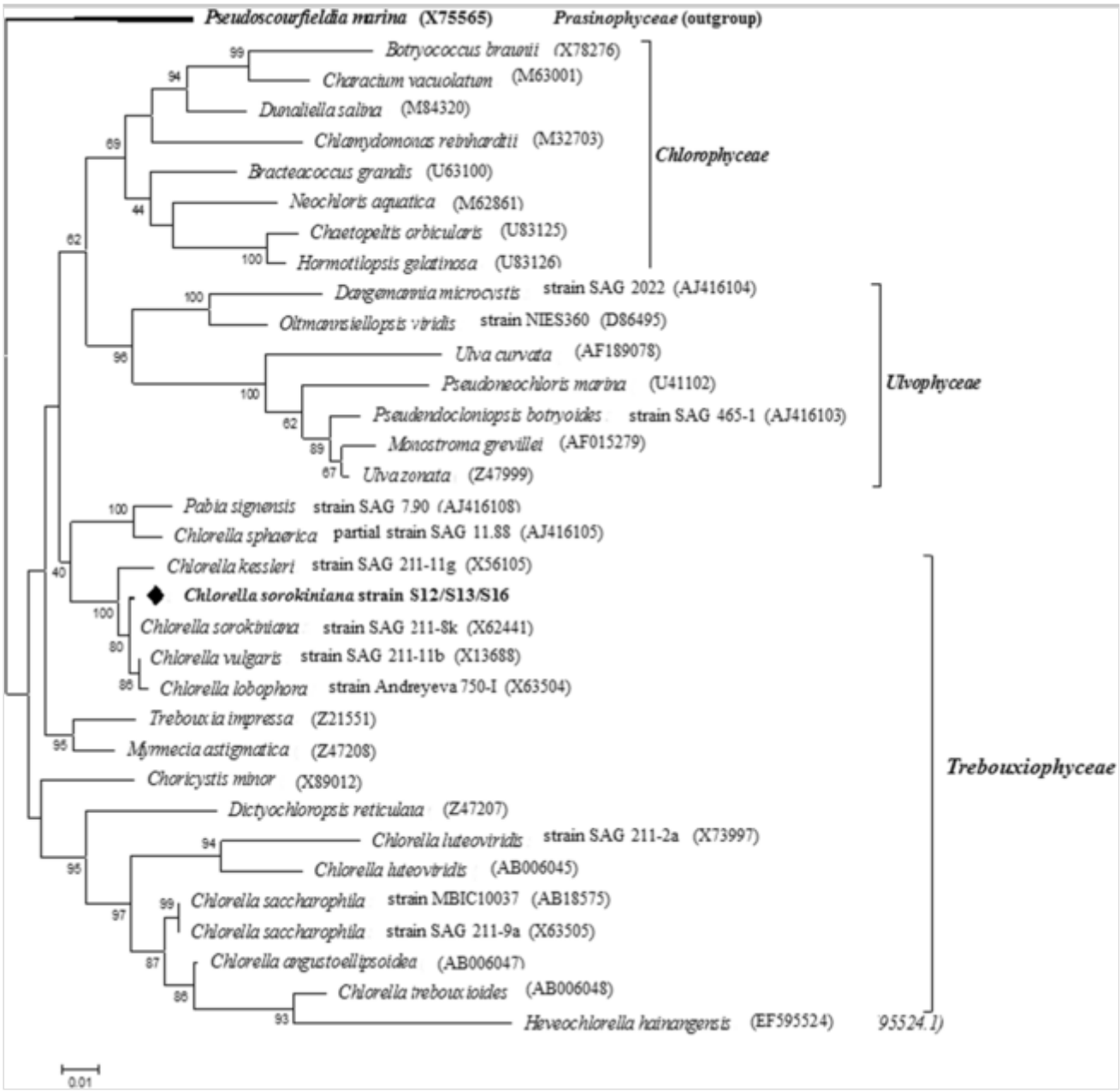
Microalgae presented in ADE medium were isolated in accordance with the procedure described in section “Microalgae isolation and selection.” Among the isolated microalgae, the fastest growth microalga was selected for identification (data not shown). This isolated microalga strain was named S12/S13/S16. The identification procedure revealed that the microalga isolated from ADE was a *Chlorella sorokiniana* strain with 99% identity. The obtained sequence was registered at GenBank database with the accession number KT852969. BLAST analysis showed that the isolated *C. sorokiniana* strain was closely related to other *Chlorella* species already registered at GenBank, including *C. sorokiniana* strain UTEX2805 (AM423162.1), *C. sorokiniana* strain GXNN01 (EU402596.1), *C. sorokiniana* strain NIES:2173 (AB731602.1), and *Chlorella* sp. ZJU0204 (JX097056.1). The results are coherent with the fact that the fastest growth microalgae species do mostly belong to *Chlorella* genus (Forján et al. 2015).

Phylogenetic analyses of 18S rRNA gene sequences demonstrated that our isolate belongs to the green algal class Trebouxiophyceae. In the 18S rRNA tree (Fig. 2), representative sequences from three major classes of the phylum Chlorophyta, Trebouxiophyceae, Chlorophyceae, and Ulvophyceae were used. The tree was rooted with one sequence from the Prasinophyceae, another chlorophytan class. *Chlorella sorokiniana* strain S12/S13/S16 clustered with four strains previously assigned to *C. sorokiniana*: *C. sorokiniana* (AM423162.1), *C. sorokiniana* (EU402596.1), *C. sorokiniana* (AB731602.1), and *C. sorokiniana* (X62441.2). Their 18S rRNA sequences share 99% identity, and they might, therefore, represent the same species. Other most closely related taxa include *C. kessleri* and *C. lobophora* with 99% bootstrap support. Since the sequence similarities between the isolated *C. sorokiniana* strain S12/S13/S16 and the most closely related strains are rather high (99%), *C. sorokiniana* is considered the same species, in agreement with morphological observations (data not shown).

Fig. 2

Phylogenetic tree of strain S12/S13/S16 and closely related species based on 18S rRNA gene sequence of ribosomal DNA. The tree was constructed using the maximum likelihood method based on the general time reversible model. The analysis involved 39 nucleotide sequences. There was a total of 1480 positions in

the final dataset. The numbers at nodes indicated the percentages of occurrence of the branching order in 1000 bootstrap trees for values greater than 50%. Scale bar = 1% divergence. The strain isolated in this study is shown in bold



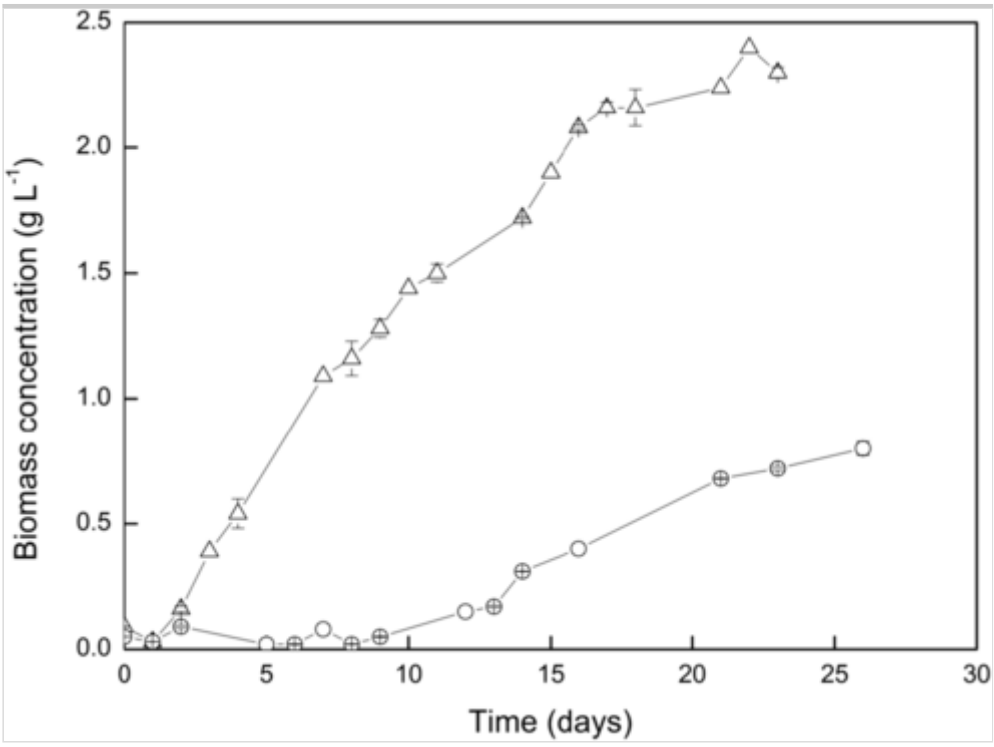
Similar work has been carried out by other authors, in which two local algal isolates (from local wastewater) were genetically characterized and used to evaluate their ability to grow and treat primary/secondary wastewater effluents as well as wastewater supplemented with nutrient-rich anaerobic digester centrate (Bohutskyi et al. 2015). As well as in this work, the two local isolates were identified as strains of *C. sorokiniana*, which is in agreement with our findings. However, main difference lies in the use of ADE effluents for the isolation in the current manuscript.

Microalgal growth in batch mode

Figure 3 shows the growth curve of the isolated *C. sorokiniana* strain *S12/S13/S16* in Sueoka medium and in ADE medium. The growth of the microalga in a non-nutrient limited Sueoka medium—without extra organic carbon added—and aerated with CO_2 should allow obtaining the maximal microalgal productivity that might be expected from that microalga cultivated under the experimental conditions of this work. Such maximal productivity should be a suitable reference to compare it with the growth efficiency of the isolated microalga in ADE medium, and it might allow identification of factors to be improved in a real biomass production later on.

Fig. 3

Growth curves of the isolated *C. sorokiniana* strain during batch cultivation, represented by the biomass dry weight evolution during the experimental time in Sueoka (triangle) and ADE media (circle). Average values are presented and error bars show the standard deviation of the different replicates



As expected, the behavior of the isolated *C. sorokiniana* grown in ADE medium was different to that in Sueoka medium (Fig. 3). In the first days, there was no growth (lag phase) in either medium. After 3 days, the microalga cultivated in Sueoka medium showed remarkable growth. In the ADE medium, an increase of microalgae concentration was observed only after 10 days. The longer lag phase in the ADE medium compared to the Sueoka medium may be attributed to the microalga adapting to the new cultivation conditions where high ammonia concentration and prokaryote organisms might be present in the ADE medium.

As explained in the "Materials and methods," CO_2-enriched air was supplied to the cultures *C. sorokiniana* in Sueoka medium, in order to avoid any carbon limitation under phototrophic conditions. Only air was supplied to the ADE culture to mimic nutrient conditions of real systems. Under the experimental cultivation conditions used, *C. sorokiniana* growth reached stationary phase at about 3 weeks after the growth started up, both in Sueoka medium and in ADE medium. However, there were significant differences in growth rate and biomass productivity between the cultures. Maximum growth rates were 0.38 day^{-1} in Sueoka medium and 0.24 day^{-1} in ADE medium. Maximum biomass productivity was 0.16 g L^{-1} day^{-1} in Sueoka medium and 0.06 g L^{-1} day^{-1} in ADE medium. As temperature and irradiance remained constant, the nutrient limitation imposed by the ADE medium, together with the presence of ammonia and the absence of an external CO_2 supply, is therefore most likely the reason for the lower growth rate in this medium. It can be explained by the chemical composition of ADE medium (Table 1), compared to the Sueoka medium, and by the absence of external CO_2 supply. Furthermore the N/P ratio in ADE medium was far above 50 (Table 1) whereas the phosphorus/P ratio of microalgal biomass is mostly below 30 (Baird and Middleton 2004; Wijffels and Barbosa 2010). Therefore, the growth of *C. sorokiniana* in ADE medium could have been limited by phosphorus, resulting in the poor growth during the batch operation (see "Inorganic nitrogen and phosphate evolution in ADE medium"). Furthermore, the consumption of phosphate from the ADE medium appeared to be linear during the experimental time. This can be explained by the restricted availability of inorganic carbon from air which might have slowed the microalgal growth rate in ADE medium. However, the supply of CO_2-enriched air is costly for wastewater treatment processes and therefore it was avoided during the experiments in order to mimic real conditions. The obtained results suggest still large potentiality of the isolated microalga for reaching high nutrient removal efficiencies and biomass productivities, which could be further enhanced if a suitable control of nutrient supply rates in a real wastewater treatment process is established.

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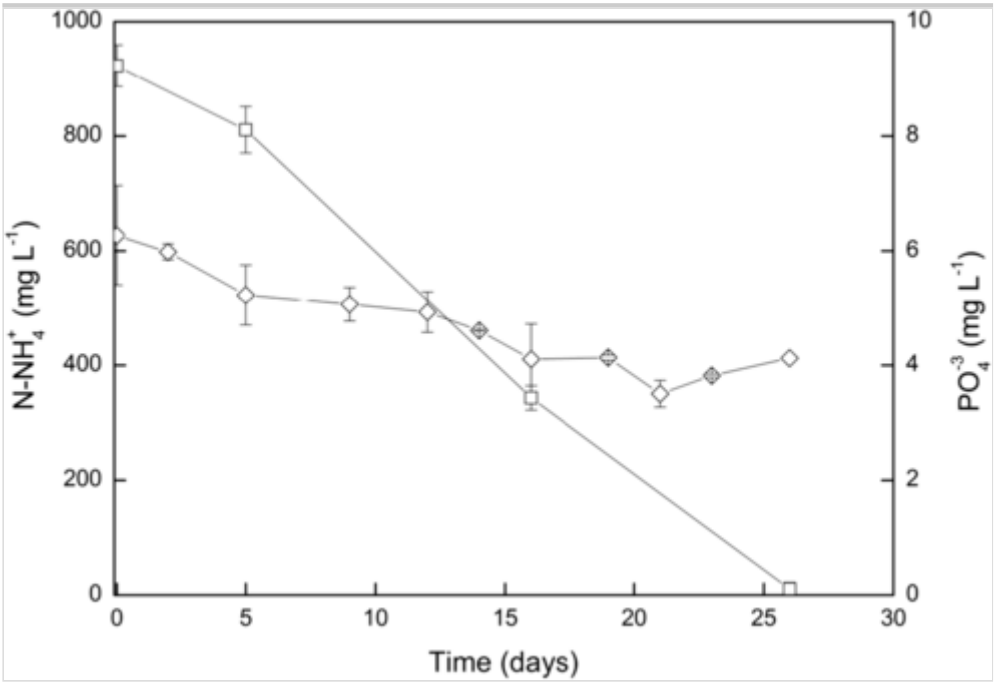
Inorganic nitrogen and phosphate evolution in ADE medium

During the batch mode cultivation in ADE medium, ammonia, nitrate, nitrite, and phosphate content were measured in the cell-free supernatant. Nitrite concentration kept very low and virtually constant throughout the cultivation process with an average value of 0.25 ± 0.06 mg $N\text{-}NO_2^-$ L^{-1}.

Figure 4 shows the time course of $N\text{-}NH_4^+$ and PO_4^{3-} concentration. Phosphate was fully removed from ADE medium at day 26, with remaining values lower than 2 mg PO_4^{3-} L^1, which accounted for about 99% of the initial PO_4^{3-} (Fig. 4). Considering the microalgal biomass produced, the obtained phosphate removal rate for *C. sorokiniana* strain S12/S13/S16 in ADE medium was 0.55 mg PO_4^{3-} g biomass day^{-1}. Such phosphorous removal rate might be even higher under non-limited growth conditions. In general terms, the phosphate removal capacity of a certain microalga depends not only on the growth conditions but also on the P-substrate affinity of a specific microalga and this may greatly differ depending on genus, species and even strains (Yamamoto et al. 2012; Kwon et al. 2013). The high phosphate removal capacity of *Chlorella* sp. has already been reported in the literature (Caporgno et al. 2015), as well as for other species like the fast-growth *Scenedesmus* sp. (Tam and Wong 1989).

Fig. 4

Time course evolution of inorganic nitrogen (ammonium) and phosphorus (phosphate) in cultures of the isolated *C. sorokiniana* strain in ADE medium, represented by the concentration in milligrams per liter of $N\text{-}NH_4^+$ (diamond) and PO_4^{-3} (square) during the experimental time. Average values are presented and error bars show the standard deviation of the different replicates



Nitrogen was mainly present in the form of NH_4^+ in the ADE medium, i.e., 91.7% of the total N present in ADE medium (Table 1). The initial $N\text{-}NH_4^+$ concentration was 626 mg $N\text{-}NH_4^+$ L^{-1}, whereas at the end of the cultivation period, the ammonium concentration was around 400 mg $N\text{-}NH_4^+$ L^{-1} (Fig. 4),

which resulted in a nitrogen removal of 36%. Higher N-NH_4^+ removal has been reported so far, above 80% (Park et al. 2010), although these reported high values were obtained without any nutrient limitation. In our experiment, the N/P ratio remained far above 50 during the entire cultivation and phosphate became exhausted after 26 days of growth. The reduced microalgal growth, imposed by the limitation of phosphorous, could explain the limited nitrogen consumption. Regarding N-NO_3^-, it accounted 8.3% of the total nitrogen present in ADE medium and it was completely removed after 20 days (data not shown). Different *Chlorella* strains have been reported to prefer ammonium as nitrogen source compared to nitrate (Li et al. 2011; Kim et al. 2013; Petrovič and Simonič 2015). Considering that, the high final ammonium concentration at the end of the cultivation period could be a good indication that nitrate removal probably was performed not only by *C. sorokiniana* but also by other nitrogen oxidizing microorganisms present in the culture medium (Mujtaba et al. 2015). In this sense, the isolated *Chlorella* strain might behave similarly to the previously reported *Chlorella* species.

C:N composition of the isolated *C. sorokiniana* biomass at the end of the culture period was 47 ± 1:8.9 ± 0.4%. At the end of the cultivation period, the biomass concentration accounted for 0.75 g L^{-1} (Fig. 3), which entailed 66.8 mg total N L^{-1} being taken up by the microalga for growth (calculated based on the final nitrogen concentration in the biomass). This nitrogen is assumed to come from the removed N-NH_4^+, considering the N-NO_3^- is not expected to be fully take up by the *C. sorokiniana* strain as previously commented. As shown in Fig. 3, ammonium removal during the experimental time was only 31% of the initial N-NH_4^+ present in the ADE medium. Considering the calculated nitrogen uptake by *C. sorokiniana* S12/S13/S16, the nitrogen removal by the biomass only accounted for 11% of the initial N-NH_4^+. Thus, the remaining N-NH_4^+ removed from ADE medium might be linked to other biological removal processes such as bacterial consumption or nitrification (Mujtaba et al. 2015). Some minor NH_3 production might have also occurred at the beginning of the experiment due to the high initial pH of ADE medium, which remained around 8.4 for 6 days (data not shown), and could explain some of the N-$NH4^+$ removal.

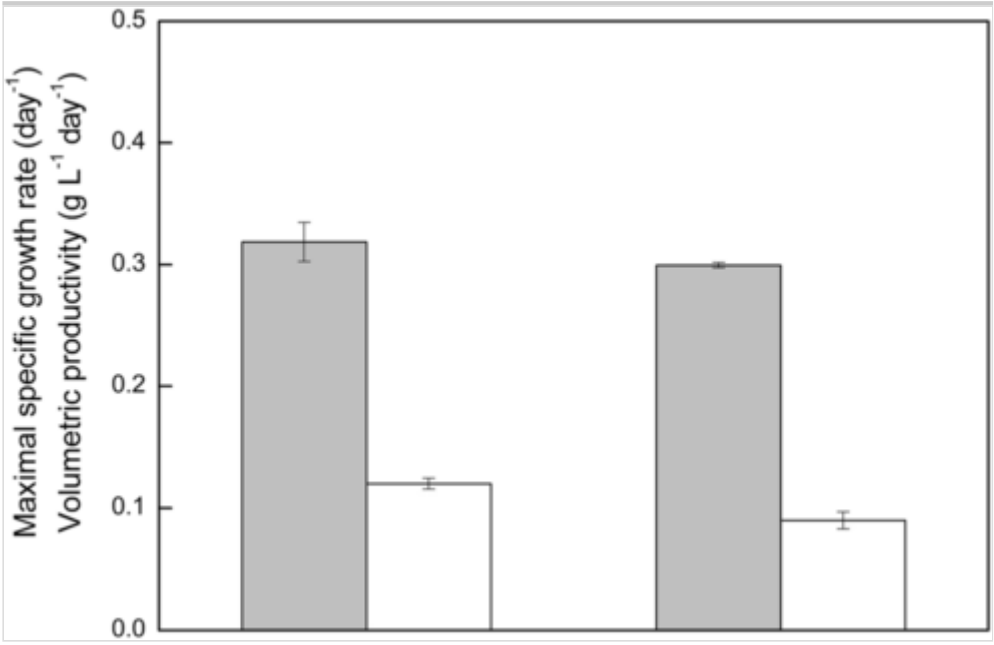
Microalgal growth in fed-batch mode

The microalgal culture grown in feed-batch mode was repeatedly diluted with ADE medium in order to maintain cellular concentration between 0.35 and 0.55 g L^{-1}. A key question at this stage of the experimental procedure was to determine the maximum productivity of *C. sorokiniana* S12/S13/S16 in ADE medium under non-limiting carbon conditions. The effect of inorganic carbon on the microalga growth was evaluated growing *C. sorokiniana* with CO_2-enriched

air, and results were compared with cultures grown exclusively with air. The volumetric biomass productivities and maximal specific growth rates obtained in the steady state during the fed-batch cultivation are shown in Fig. 5. As expected, under the studied conditions, volumetric productivity and growth rate of *C. sorokiniana* cultures in ADE medium with CO_2-enriched air were slightly higher than those cultures supplied with air (Fig. 5). However, the biomass productivity obtained in cultures supplied with CO_2-enriched air ($0.12\ g\ L^{-1}\ day^{-1}$) was only a 33% higher than that obtained in cultures with air only. Under that scenario where microalgal growth seems to be limited by phosphorous and there is no CO_2 available, the observed increase in biomass productivity seems not to justify the extra cost of CO_2 supply. However, there are other scenarios where CO_2 is available (i.e., by burning biogas produced in the wastewater plant) and therefore it could be easily employed in the microalgal cultivation resulting in better productivities.

Fig. 5

Maximal specific growth rate (gray bars) and biomass productivity (white bars) of the isolated *C. sorokiniana* strain cultivated in ADE medium in fed-batch mode with and without the external addition of CO_2. Average values are presented, and error bars show the standard deviation of the different replicates



Compared to previous studies, the productivities reported for other *C. sorokiniana* strains in wastewater supplemented with 5–10% nutrient-rich anaerobic digestion centrate were lower ($0.06\ g\ L^{-1}\ day^{-1}$) than those in this study, although these values could be improved when using only the anaerobic digestion centrate ($0.2–0.6\ g\ L^{-1}\ day^{-1}$) (Bohutskyi et al. 2016). Furthermore,

the maximum biomass productivity obtained by fed-batch cultivation in ADE medium was similar to productivity values reported for several microalgal strains cultivated in different waste streams under the fed-batch regime and without CO_2 supplementation (0.15–0.21 g L^{-1} day^{-1}) (Xia and Murphy 2016).

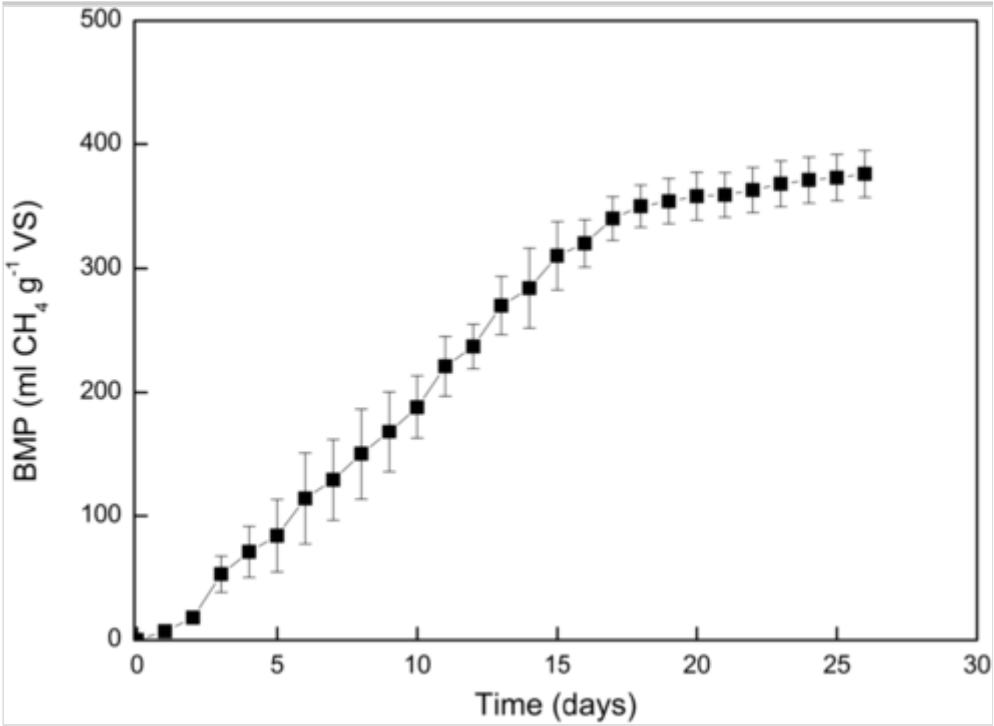
Maximum specific growth rates were little different (6% higher in cultures with CO_2-enriched air). The similar values obtained might be explained by the nutrient limitation imposed by the ADE medium, as well as the by light limitation imposed by the experimental design, and not by the absence of inorganic carbon. Consequently, it can be inferred that the overproduction of algal biomass in ADE medium under CO_2-saturating conditions would not always compensate the extra cost of CO_2 and therefore it should be particularly addressed for every scenario.

Potential energy recovery from the produced microalgae

The obtained microalgal biomass is proposed to be energetically valorized being fed in a sewage sludge anaerobic digester (Fig. 1). In this sense, the BioMethane Potential (BMP) of *C. sorokiniana* S12/S13/S16 was measured (Fig. 6). It can be observed that *C. sorokiniana* reached values of about 370 mL CH_4 g^{-1} VS. These values are similar to those reported in the literature for other microalgae (Muñoz et al. 2014; Fermoso et al. 2016). From an energetic point of view, if the energy produced in the hypothetical methane combustion is computed, anaerobic digestion of microalgae should produce about 13.2 MJ kg^{-1} VS. This energy could be re-used in the process, not only for the wastewater treatment plant but also to supply energy requirements for microalgal cultivation and for harvesting. Both thermal and electrical energy could be obtained if biogas combustion in co-generation systems is considered, as computed by Torres et al. (2015). The *C. sorokiniana* methanogenic potential together with its robust growth makes it a good candidate for integrated processes of nutrients recovery from wastewaters and biomass anaerobic digestion.

Fig. 6

Accumulated methane production over time of anaerobic digestion of the isolated *C. sorokiniana* biomass. Average values are presented, and error bars show the standard deviation of the different replicates



Conclusions

A robust microalgae belonging to *Chlorella* genus was isolated from the anaerobic digestion effluent. *Chlorella sorokiniana* S12/S13/S16 growth in ADE was limited by phosphorus, which reached removal efficiency close to 100%. However, nitrogen removal was only 31% of the N-total, most probably due to P limitation. Biomass productivity and growth rate were slightly enhanced with CO_2 supply, but this would not compensate the cost. Use of ADE as nutrients source for the cultivation of local microalgal strains might become a suitable and sustainable treatment. Biomethane production could contribute to fulfill energy requirements for the proposed whole system.

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