Nutrient recycling from the effluent of a decentralized anaerobic membrane bioreactor (AnMBR) treating fresh domestic wastewater by cultivation of the microalgae *Acutodesmus obliquus*

M. Foix-Cablé, R. A. Darmawan, M. Sahnoun, S. Hindersin, M. Kerner and M. Kraume

ABSTRACT

This study investigates the feasibility of microalgae cultivation with the effluent (permeate) of a decentralized anaerobic membrane bioreactor (AnMBR) treating high strength domestic wastewater. Two experiments, consisting of three and two successive batch experiments with incubation times varying between 5 and 9 days, were conducted. Nutrient removal and growth of the microalgae species *Acutodesmus obliquus* were studied for the following culture media: (A) permeate, (B) permeate enriched with iron (Fe), magnesium (Mg), manganese (Mn), sulfur (S) and the chelating agent EDTA, (C) commercial fertilizer as control culture. Initial nutrient concentrations in the culture media ranged from 9.3 to 16.6 mg·L^{-1} total phosphorus (TP) and from 85.1 to 126.2 mg·L^{-1} total nitrogen (TN). TP reached an average removal of 97%, 98% and 99% in (A), (B) and (C) respectively. An average TN removal of 94% and 96% was achieved in (B) and (C). Starting from the third batch of the first experiment and the second batch of the second experiment, the culture with permeate (A) showed a decrease in TN removal. Further batch experiments showed the need to add iron to ensure an optimal TN removal from the permeate.

Key words | anaerobic membrane bioreactor, microalgae, nutrient removal, wastewater treatment, water recycling

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INTRODUCTION

Domestic wastewater treatment with efficient nutrient recovery is a worldwide key challenge for the 21st century. While industrialized countries treat 70% of their sewage, between 28% and 38% of the wastewater is treated in the emerging countries and only 8% in the developing countries (Sato *et al.* 2013). When the wastewater is treated, nitrogen and phosphorus removal often remains insufficient, leading to eutrophication of the water bodies (Rawat *et al.* 2011). At the same time, phosphorus resources are expected to be depleted within the next 100 years (Withers *et al.* 2015) and demand could exceed supply starting from 2035 (Cordell 2010). Phosphorus from human feces represents 28% of the phosphorus global demand (Mihelcic *et al.* 2011). If this major nutrient was reused, it could lead to an enhancement of the water bodies' quality and be a long-term solution to phosphorus depletion. For

this purpose, microalgae cultivation appears to be well adapted:

- Contrary to conventional wastewater treatments, nitrogen and phosphorus can be entirely and simultaneously removed without the addition of any chemical product. At the same time, these main nutrients are recycled through biomass production.
- Microalgae do not need an external organic carbon source. During photosynthesis, they instead use carbon dioxide, one of the main gases responsible for the global warming, as a carbon source.
- Numerous high value products are made from microalgae. They include especially lipids, proteins, pigments and fatty acids (Spolaore *et al.* 2006). Nevertheless, the most promising application for microalgae grown on

wastewater effluents is their use for the purpose of biofuel production (Olguín 2012).

- Coupling wastewater treatment and microalgae cultivation enables fertilizer to be saved based on limited resources, given that the production of 1 kg of fertilizer can generate up to 3.47 kg of carbon dioxide (Brentrup *et al.* 2010).

Besides, in the field of wastewater treatment, the use of AnMBRs has multiplied in the recent years. The low amount of sludge produced, the small footprint and the high quality solid free effluent obtained in a one-unit process are the main advantages of AnMBRs over conventional aerobic processes (Lin *et al.* 2013; Ozgun *et al.* 2013). However, this process implicates the need for a post-treatment to eliminate the nitrogen and phosphorus (Smith *et al.* 2012).

The present work aims to recycle the nutrients contained in the effluent of a decentralized AnMBR treating fresh domestic wastewater by coupling this preliminary process with the production of the microalgae species *Acutodesmus obliquus*. Even though research in the field of nutrient recovery from wastewater streams by means of microalgae culture has intensified over the past ten years, coupling an AnMBR with microalgae cultivation is a comparatively new area of study. Regarding microalgae growth, this effluent differs from the effluents of other anaerobic systems, as it is solid free and usually characterized by a lower turbidity that enhances photosynthetic activity. Total nitrogen (TN) and total phosphorus (TP) are not present in the form of particles and only consist of ammonium and phosphates, which are the preferential forms of nitrogen and phosphorus for the microalgae.

Ruiz-Martinez *et al.* (2012) showed that a submerged anaerobic membrane bioreactor (SAnMBR) treating municipal wastewater was suitable for continuous microalgae growth. Viruela *et al.* (2016) demonstrated the feasibility of using an AnMBR treating pre-treated sewage in combination with microalgae production for nutrient removal. However, to our knowledge, coupling a decentralized AnMBR with microalgal culture has never been performed. Municipal wastewater used in previous studies originated from centralized wastewater treatment plants. During the transport to the wastewater treatment plant, this sewage is diluted with rainwater, street cleaning water and low strength industrial wastewater. In contrast, domestic wastewater from decentralized systems is usually characterized by much higher nutrient concentrations.

In this study, the influence of the addition of the micronutrients Fe, Mg, Mn and S was simultaneously investigated.

Indeed, in addition to carbon, nitrogen and phosphorus, numerous micronutrients are required at low amounts to obtain maximum biomass production (Grobbelaar 2004). While the effects of micronutrients on microalgae growth are well documented, information about their availability in wastewater effluents used for microalgae culture is hardly found. Tuantet *et al.* (2014) stated the necessity of adding Mg to nutrient-rich human urine to reach higher biomass productivities. However, to our knowledge, no literature about the influence of micronutrient addition to the effluent of an anaerobic system treating wastewater is available.

METHODS

Organization of the experiments and culture media

This work was divided into five experiments. Experiment (1) focused on the performance of permeate regarding microalgae growth and TN and TP removal compared to a commercial fertilizer. To assess the influence of additional micronutrients on this performance, the three following culture media were investigated:

- (A) Permeate from the AnMBR.
- (B) Permeate enriched with the elements Mg, Mn, Fe, S and EDTA. Using magnesium sulfate ($MgSO_4 \cdot 7H_2O$), manganese (II) chloride ($MnCl_2 \cdot 4H_2O$) and iron (II) sulfate ($FeSO_4 \cdot 7H_2O$), $7\ mg \cdot L^{-1}$ of Mg, $5\ mg \cdot L^{-1}$ of Mn, $25\ mg \cdot L^{-1}$ of Fe and $23\ mg \cdot L^{-1}$ of S were added. The chelating agent EDTA, which favors the formation of complexes with the micronutrients and, therefore, their assimilation by microalgae, was added with an initial concentration of $8\ mg \cdot L^{-1}$. These concentrations were chosen to ensure that the micronutrients would not become limiting factors during the experiments. Hence, they were similar to or higher than in the commercial fertilizer and the culture media usually used for microalgae culture. EDTA was added following the composition of the KC medium (Kessler & Czygan 1970).
- (C) A commercial fertilizer (Ferty Basis 1, Planta Düngemittel GmbH, Germany) was used as control for the experiment. As it does not contain a nitrogen source, nitrogen was added in the form of ammonium nitrate (NH_4NO_3). NH_4NO_3 was preferred to an ammonium salt, as the culture media usually reach high levels of acidity when ammonium is the only source of nitrogen and nitrate can be assimilated by the microalgae and

reduced to ammonium. The commercial fertilizer was diluted with distilled water and NH_4NO_3 added so that TN and TP initial concentrations were similar to those of the cultures (A) and (B). Based on the composition of the commercial fertilizer, the initial micronutrient concentration amounted to 6 mg·L^{-1} of Mg, 25 mg·L^{-1} of S, 0.4 mg·L^{-1} of Fe and 0.1 mg·L^{-1} of Mn. Fe was present in the commercial fertilizer as chelate from DTPA and EDDHA and Mn as chelate from EDTA.

The further experiments (2), (3), (4) and (5) focused on the confirmation and explanation of the results obtained with the permeate (A) and the enriched permeate (B) during the experiment (1). In experiment (2), experiment (1) was reproduced with the culture media (A) and (B). Using magnesium chloride, manganese (II) chloride, iron (II) sulfate and EDTA, the micronutrients and the chelating agent concentrations were similar to (1).

After the second batch test of (2), the culture medium with permeate (A) was divided into two triplicates for experiment (3). On day 0 of (3), $MnCl_2 \cdot 4H_2O$ and EDTA were added into three bottles (culture medium (D)) and $MgCl_2 \cdot 7H_2O$ and EDTA into the three other bottles (culture medium (E)). After 2 days, $FeSO_4 \cdot 7H_2O$ was added into the culture medium (D) and $MnCl_2 \cdot 4H_2O$ into the culture medium (E).

Experiment (4) aimed to confirm the results obtained in (3). A culture composed of new permeate and microalgae (A) from the end of experiment (2) was started in triplicates. On day 5 of (4), the culture was divided into six bottles. $MgCl_2 \cdot 7H_2O$, $MnCl_2 \cdot 4H_2O$, $FeSO_4 \cdot 7H_2O$ and EDTA were added in one triplicate and $FeSO_4 \cdot 7H_2O$ and EDTA in the other triplicate.

During experiment (5), *Chlorella vulgaris* was cultivated throughout three batch experiments with permeate (A) and commercial fertilizer (C) in order to verify the results observed with *Acutodesmus obliquus*. At the end of the third batch, the culture (A) was divided into six bottles. $FeSO_4 \cdot 7H_2O$ and EDTA were added in one triplicate and $FeCl_2 \cdot 4H_2O$ and EDTA in the other triplicate.

The addition of micronutrients in experiments (3), (4) and (5) was similar to experiment (2).

Effluent from the AnMBR

A prototype AnMBR was built and automated at a residential building (approximately 30 inhabitants) called 'BIQ The Algae House' in Hamburg, Germany. Fresh and untreated wastewater produced in the building was pumped to an 800 litre reactor (Figure 1), where organic compounds were digested under anaerobic and mesophilic conditions (~37 °C). The permeate was obtained by means of a crossflow external ultrafiltration membrane (Bioflow, 30 nm pore size, Berghof, Germany). At the beginning of each experiment, new permeate was sampled once for all batches and stored at 4 °C.

Experimental set-up

The experimental plant for experiment (1) consisted of nine autoclaved tubular pipes with a capacity each of 350 mL (three pipes for each culture medium). The pipes were submerged in a water bath at 25 °C (Thermostat DC40-K40, Haake, Germany). Five neon tubes (Philipps, Master TL-D 58 W/840) gave an average light intensity of 446 ± 44 µmol·s^{-1}·m^{-2}. The experimental set-up worked with a 14:10 h light/dark cycle. A continuous circulation of air and carbon dioxide (1 L·min^{-1}·$L_{culture}^{-1}$) with a carbon dioxide concentration from 4% to 5% was used. As pH values in the range 7–9 are reported to be optimal for microalgal growth and a pH kept to neutrality allows NH_4^+-N to be the sole form of ammonia nitrogen (Ruiz-Martinez *et al.* 2012), pH (pH 91, WTW, Germany) was set to 7.5. Every day, pH was controlled and, when the pH was lower than 7, adjusted with sodium hydroxide.

During the further experiments, microalgae were cultured in 1-L Schott bottles. The cultures were fumigated with carbon dioxide (0.05 L·min^{-1}·$L_{culture}^{-1}$) and room air (0.95 L·min^{-1}·$L_{culture}^{-1}$). The culture media were mixed using magnetic stirrers. The microalgae were continuously illuminated by two full spectrum LED daylight tubes (Naturnah, 26 W, Germany). The illumination corresponded to 270 ± 36 µmol·s^{-1}·m^{-2}. During the experiments, the pH
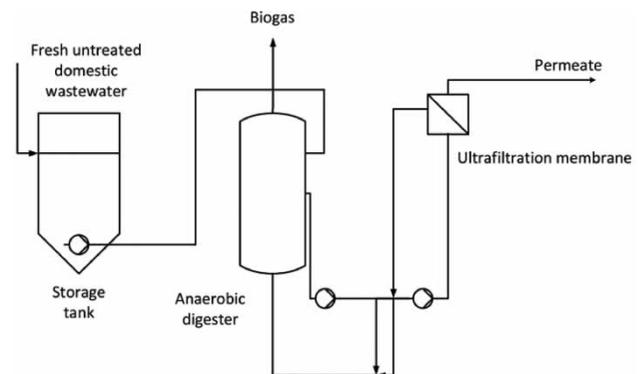

Figure 1 | Simplified flow diagram of the decentralized AnMBR process.

remained at a neutral level (7.0 ± 0.1) and the temperature of the cultures amounted to 23 ± 1 °C.

Microalgae pre-culture and inoculation

For the experiment (1), 20 mL *Acutodesmus obliquus* from the microalgae collection MCZH-SVCK of the University Hamburg was used as pre-culture medium. As TP removal is commonly lower than TN removal during microalgae cultivation and *Acutodesmus obliquu*s generally leads to higher TP removal than other microalgae species (Whitton *et al.* 2015), the use of this species was preferred. The pre-culture was cultivated in a 2 litre Duran bottle in presence of a diluted commercial fertilizer (Ferty Basis 1, Planta Düngemittel GmbH, Germany). Before starting the pre-culture, the culture medium was autoclaved and pH was adjusted to 7.5. Continuous lighting of 331.7 $\mu mol \cdot s^{-1} \cdot m^{-2}$ was applied. After 7 days, the pre-culture was centrifuged for 10 min at 4,000 rpm (Thermo Scientific, USA). The biomass was then washed with distilled water and centrifuged again to obtain algae cells free of nutrients contained in the culture medium. The algae cells obtained were subsequently added at equal mass into the nine pipes and inoculated with the culture media. 10 μL of silicon anti-foaming agent was added in each pipe in order to avoid the formation of foam and the loss of the biomass. To start a new batch experiment, the contents of the three pipes of each culture were mixed together in a 2 litre Duran laboratory bottle. After 10 min centrifugation at 4,000 rpm, the biomass was divided in the same three pipes and inoculated with the corresponding culture medium.

In experiment (2), microalgae culture from a 60 L outdoor photobioreactor cultivating *Acutodesmus obliquus* were used for the inoculation. The inoculation procedure was the same as for experiment (1).

In experiment (5), 20 mL *Chlorella vulgaris* from the microalgae collection MCZH-SVCK of the University Hamburg was used as pre-culture medium. The pre-culture and inoculation procedures were the same as for experiment (1).

Analysis

For biomass concentration determination, the samples were vacuum filtrated (cellulose membrane, 0.45 μm, Sartorius, Germany) and dried in a drying furnace (Heraeus, Germany) at 80 °C for 24 hours. The biomass concentration

was obtained as follows:

$$C = (W_{total} - W_{filter})/V_{sample}$$

where C is the biomass concentration (g·L^{-1}), W_{total} is the weight of the dried sample (g), W_{filter} is the initial weight of the filter (g) and V_{sample} is the volume of the sample (L).

The biomass production rate was calculated under limiting light conditions with the following equation:

$$BPR = (C_f - C_i)/(t_f - t_i)$$

where BPR is the biomass production rate (g·L^{-1}·d^{-1}), C_i and C_f are the initial and final biomass concentrations (g·L^{-1}) and t_i and t_f are the starting day and the final day of the batch experiment.

After filtration of the cultures, standard cuvette tests from the company Hach Lange were used for NH$_4^+$-N and TP measurements of the culture media. TN was analyzed with the TOC-VCPN/TNM-1-Analyser (Shimadzu, Japan) for experiment (1) and with Hach Lange standard cuvette tests during the further experiments.

RESULTS AND DISCUSSION

Experiment (1) - biomass growth

The first batch experiment showed a similar growth for cultures (A) and (B), which reached a biomass concentration of respectively 2.32 g·L^{-1} and 2.45 g·L^{-1} (Figure 2) after 8 days. The microalgae cells of experiment (C) only reached a final biomass concentration of 1.36 g·L^{-1} due
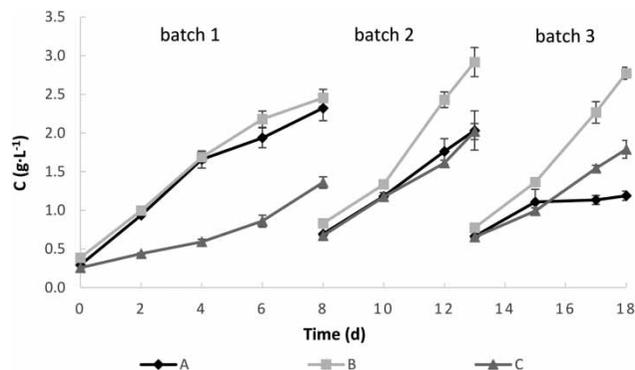

Figure 2 │ Biomass concentration throughout three batch experiments using permeate (A), enriched permeate (B) and commercial fertilizer (C) as culture media during the experiment (1). The error bars represent the standard deviations of the triplicates.

to daily decreases of pH (see supplementary material, available with the online version of this paper). During this first experiment, BPR of 0.253, 0.258 and 0.138 $g \cdot L^{-1} \cdot d^{-1}$ were achieved for the cultures (A), (B) and (C) respectively.

During the second batch experiment, cultures (A) and (C) had similar growth, reaching a final biomass concentration of 2.03 and 2.02 $g \cdot L^{-1}$ respectively after 5 days. The BPR amounted to 0.268 $g \cdot L^{-1} \cdot d^{-1}$ in (A) and 0.269 $g \cdot L^{-1} \cdot d^{-1}$ in (C). Culture (B) performed better growth and achieved a final biomass concentration of 2.91 $g \cdot L^{-1}$ and a BPR of 0.417 $g \cdot L^{-1} \cdot d^{-1}$.

During the third batch experiment, the differences between cultures (A) and (B) intensified. Culture (B) continued to grow well and achieved a BPR of 0.400 $g \cdot L^{-1} \cdot d^{-1}$. On the contrary, microalgae growth in permeate (A) was almost non-existent. The BPR reached 0.105 $g \cdot L^{-1} \cdot d^{-1}$, which represented only 26% of the BPR obtained with culture (B). The final biomass concentration in (B) reached 2.77 $g \cdot L^{-1}$ and was 132% and 55% higher than in (A) and (C) respectively.

Experiment (1) – nutrient removal

During the first batch experiment, 4 days were needed to remove 93% of TN in cultures (A) and (B) (Figure 3). During the second batch experiment, it also took 4 days to remove 96% of TN in (A) and 96% of TN in (B). Starting from the third batch experiment, microalgae were not able any more to totally assimilate the TN content of the culture (A). After 5 days, the removal efficiency only achieved 58%. At the same time, 96% of the TN was removed from the culture (B). In control culture (C), TN removal efficiencies of 93%, 98% and 96% were achieved after 8, 5 and 5 days respectively.
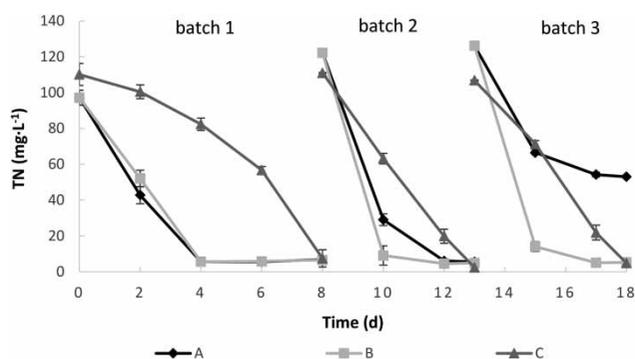
TN results were confirmed by NH_4^+-N removal results (see supplementary material). During the first two batch experiments, NH_4^+-N was entirely removed from each culture with removal efficiencies of 100%. During the third batch experiment, NH_4^+-N removal achieved likewise 100% in cultures (B) and (C), whereas it only reached 59% in culture (A). In culture (A), NH_4^+-N final concentrations amounted to 0.12, 0.13 and 40.7 mg NH_4-N$\cdot L^{-1}$. In cultures (B) and (C), NH_4^+-N remained only in very low concentrations varying between 0.07 and 0.11 mg NH_4-N$\cdot L^{-1}$ in (B) and between 0.05 and 0.16 mg NH_4-N$\cdot L^{-1}$ in (C).

With regard to TP, 97%, 94% and 99% removal were reached in permeate (A) and 96%, 97% and 99% in the enriched permeate (B) (Figure 4). At the end of each batch experiment, TP was present only in very low concentrations varying in the range of 0.11 and 0.88 $mg \cdot L^{-1}$. These results were similar to the results obtained with the control culture (C), where TP achieved final concentrations between 0.05 and 0.26 $mg \cdot L^{-1}$ and removal efficiencies of 98%, 98% and 100%.

Experiment (2) – TN, TP, NH_4^+-N removal and microalgae growth

During the first batch experiment of (2), very similar results were obtained (Figures 5(a) and 5(b)). TN, NH_4^+-N and TP removal amounted to 95%, 100% and 98% in (A) and 92%, 100% and 99% in (B). Starting from the second batch experiment, a decrease of TN removal in permeate (A) occurred. While the microalgae in (B) eliminated 91% of TN and 100% of NH_4^+-N, TN and NH_4^+-N removal in (A) only reached 58% and 71%. A high TP removal was achieved in both culture media (96% for (A) and 98% for

Figure 3 │ TN concentration throughout three batch experiments using permeate (A), enriched permeate (B) and commercial fertilizer (C) as culture media during the experiment (1). The error bars represent the standard deviations of the triplicates.
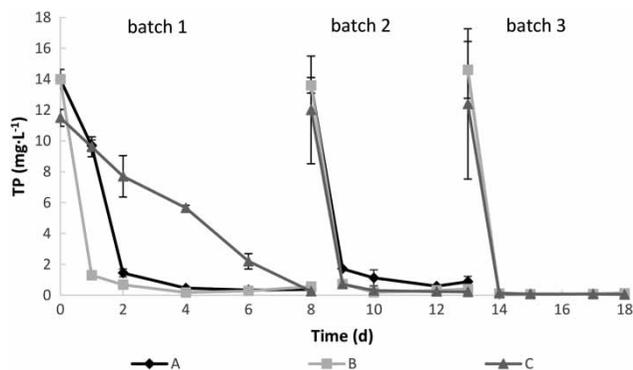
Figure 4 │ TP concentration throughout three batch experiments using permeate (A), enriched permeate (B) and commercial fertilizer (C) as culture media during the experiment (1). The error bars represent the standard deviations of the triplicates.
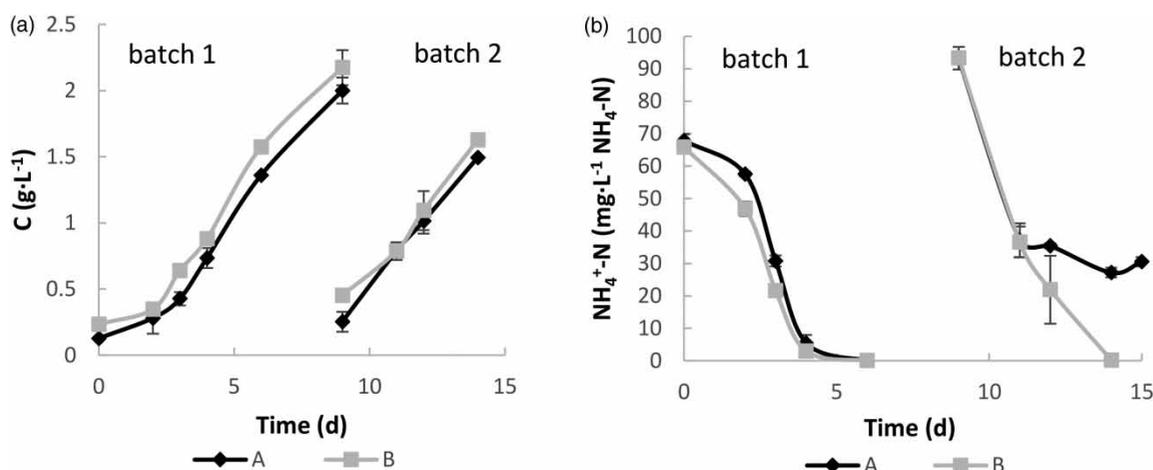
Figure 5 | Biomass (a) and ammonium (b) concentrations throughout two batch experiments using permeate (A) and enriched permeate (B) as culture media during the experiment (2). The error bars represent the standard deviations of the triplicates.

(B)). Contrary to experiment (1), no decrease in biomass production was observed in permeate (A) compared to the enriched permeate (B). The BPR reached 0.25 g·L^{-1}·d^{-1} in (A) and 0.23 g·L^{-1}·d^{-1} in (B).

Experiments (3) and (4) – influence of micronutrient addition to the permeate (A)

During the experiment (3), the microalgae culture (A) from the end of experiment (2) was enriched with the micronutrients mentioned in the Methods section. After 2 days, TN remained at a mean concentration of 56.9 mg·L^{-1} in the culture enriched with Mn and EDTA (D) and 54.6 mg·L^{-1} in the permeate enriched with Mg and EDTA (E) (see supplementary material). After the addition of Mn to the culture medium (E), TN remained stable at 54.5 mg·L^{-1}. However, the addition of FeSO$_4$·7H$_2$O to the culture (D) led to a decrease in the TN concentration by 68% after 2 days. In this experiment, it appears that the addition of iron (II) sulfate was responsible for the improved TN

removal observed in the culture (B) during the experiments (1) and (2).

The experiment (4) aimed to confirm the results observed during the experiment (3). In the culture containing new permeate inoculated with microalgae from the culture medium (A) of the experiment (2), the TN concentration ceased to decrease starting from the third day and reached a final concentration of 41.3 mg·L^{-1} (Table 1). On the fifth day, the contents of the three bottles were divided into six bottles and the different micronutrients were added as defined under methods. After 2 more days, the NH$_4^+$-N concentration was very low in each culture medium. TN reached a final value of 14.8 mg·L^{-1} in the culture enriched with Fe, S, Mn, Mg and EDTA and 12.1 mg·L^{-1} in the culture enriched with Fe, S and EDTA. These results showed that only the addition of iron (II) sulfate was responsible for the great amelioration of TN removal in the permeate culture. On the contrary, Mg and Mn addition did not play a role in TN removal. However, the final biomass concentration was slightly better in

Table 1 | TN, NH$_4^+$-N and biomass concentrations during the experiment (4)

Culture medium	Duration (days)	NH$_4^+$-N concentration (mg NH$_4$-N·L^{-1})		TN concentration (mg·L^{-1})		Biomass concentration (g·L^{-1})	
		Initial	Final	Initial	Final	Initial	Final
Permeate	5	62.1 ± 7.6	30.5 ± 1.6	104.3 ± 1.7	41.3 ± 1.9	0.20 ± 0.03	1.51 ± 0.07
Addition of Fe, Mg, Mn, S and EDTA after 5 days	2	23.5 ± 1.4	0.073 ± 0.001	–	14.8 ± 1.6	1.58 ± 0.08	4.77 ± 0.49
Addition of Fe, S and EDTA after 5 days	2	24.2 ± 3.2	0.067 ± 0.017	–	12.1 ± 0.8	1.73 ± 0.12	3.90 ± 0.37

The errors represent the standard deviations of the triplicates.

permeate enriched with Fe, S, Mn, Mg and EDTA (4.8 g·L^{-1}) than in permeate enriched with Fe, S and EDTA (3.9 g·L^{-1}).

Experiment (5) – results verification by cultivation of *Chlorella vulgaris* – iron function in TN removal

By cultivating *Chlorella vulgaris* with permeate, similar results regarding TN removal were observed (Figure 6). During the first two batch tests, NH$_4^+$-N was entirely assimilated by the microalgae and final concentrations of 0.052 and 0.064 mg·L^{-1} were achieved. TN removal reached 93% and 91% and was similar to the commercial fertilizer. During the third batch test, only 61% of TN was removed from permeate (A) after 6 days. Simultaneously, 92% of TN removal was achieved in commercial fertilizer (C) after 2 days. Whereas TP was entirely removed throughout the three batches, the BPR in (A) only amounted to 52% and 44% of the BPR in (C) in the second and third batch tests (see supplementary material). Hence, the results observed with *Acutodesmus obliquus* were confirmed with *Chlorella vulgaris*, and the decrease of TN removal and biomass production in the permeate media (A) was not due to the use of the species *Acutodesmus obliquus*.

Fe is one of the most important micronutrients for microalgae growth and is especially a key element in TN assimilation (Naito *et al.* 2005; Quigg 2016). Therefore, it was assumed that Fe had a very important function in the increase of TN removal observed by addition of iron (II) sulfate to the permeate. In order to assess the function played by Fe in TN removal, the culture was divided into two triplicates at the end of the third batch test of (5). Iron (II) chloride and iron (II) sulfate were added to the different triplicates. In both triplicates, NH$_4^+$-N concentration was very similar and lower than 0.58 and 0.02 mg·L^{-1} after 2 and 4 days respectively. TN reached final concentrations of 6.97 mg·L^{-1} in permeate enriched with iron (II) sulfate and 7.47 mg·L^{-1} in permeate enriched with iron (II) chloride. Hence, only the lack of Fe was responsible for the decrease in TN removal observed in permeate cultures (A) during the experiments (1), (2), (4) and (5).

Performance of the permeate enriched with micronutrients and EDTA (B) compared to the commercial fertilizer (C)

During the experiment (1), nutrient uptake occurred faster with enriched permeate (B) than with commercial fertilizer (C) and the microalgae in (B) showed higher BPR. These differences were certainly caused by the great pH fluctuations in the culture (C). Indeed, despite a daily adjustment of the pH close to neutrality, very acidic levels (pH 3–5) were reached during the first 2 days of each batch experiment (see supplementary material). The pH value is known to be one of the most critical environmental conditions (Qiu *et al.* 2017). Extreme pH values cause direct physiological effects on the microalgae cells (Chen & Durbin 1994) and a rapid acidification of the medium can even cause cell death (Lee & Zhang 2014). In the present study, the permeate showed better buffer capacities, which
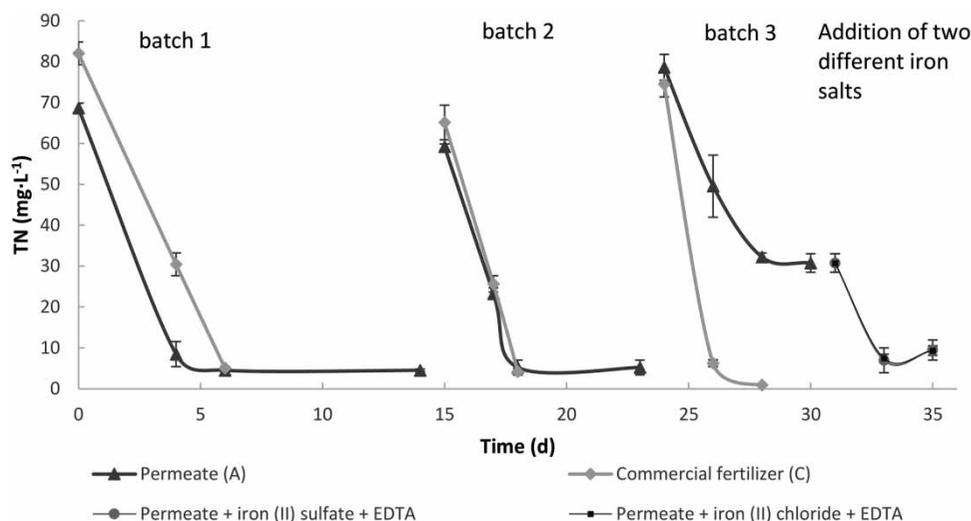

Figure 6 │ TN concentration throughout three repeated batch experiments using permeate (A) and commercial fertilizer (C) as culture media during the experiment (5) conducted with *Chlorella vulgaris* and comparison of TN removal by addition of iron (II) sulfate + EDTA and iron (II) chloride + EDTA to the permeate at the end of the third batch test. The error bars represent the standard deviations of the triplicates.

lead to stabilized pH values during NH_4^+-N uptake by the microalgae. This allowed microalgae cultures without external pH regulation. However, the slower nutrient uptake and microalgal growth in the control (C) could also be explained by the higher amount of energy needed by the microalgae to assimilate nitrate. Indeed, ammonium nitrate was used as nitrogen source. Nitrate uptake required reduction to NH_4^+-N involving the use of enzymes in the microalgae cells, and this reaction could have influenced the TN uptake rate and the BPR of the control culture (C).

Nutrient removal in the enriched permeate (B) compared to literature

Regarding nutrient removal, the results of the present work were similar to Martínez *et al.* (2000), who found a removal of 98% TP and 100% NH_4^+-N during the cultivation of *Acutodesmus obliquus* with wastewater from a conventional secondary treatment facility under similar experimental conditions and reaction times. Using the same species, Gupta *et al.* (2016) obtained similar results and achieved 98.54% TN removal and 97.99% TP removal with raw urban sewage. In the present work, nutrient removal was much higher than in previous experiments conducted with effluents of AnMBRs or SAnMBRs. Ruiz-Martinez *et al.* (2012) succeeded in removing 97.8% of phosphate from the effluent of an AnMBR treating pre-treated domestic wastewater. However, on average, only 67.2% of NH_4^+-N was removed. This low removal efficiency was due to fluctuating NH_4^+-N concentrations in the effluent of the AnMBR (42.6–81.4 mg·L^{-1} NH_4-N) combined with a stable hydraulic retention time of 2 days. With the effluent of an AnMBR fed with pre-treated sewage, Viruela *et al.* (2016) achieved NH_4^+-N removal in the range of 36.3–75.2% and phosphate removal in the range of 36.1–77.9%. The great fluctuations of NH_4^+-N and phosphate concentrations in the effluent (40–100 mg·L^{-1} NH_4-N and 6–11 mg·L^{-1} PO_4-P) could explain the lower nutrient removal. Furthermore, three 550 litre outdoor photobioreactors were used for this experiment, so the strong dependence on the weather conditions might explain this discrepancy.

Influence of the use of high strength domestic wastewater as substrate of the AnMBR

A further challenge in this work was the high concentration of TN and TP in the permeate compared to municipal wastewater from centralized wastewater treatment plants. Whereas Whitton *et al.* (2015) reported NH_4^+-N and TP

concentrations in the range of 0.1–35.5 mg NH_4-N·L^{-1} and 0.04–11.8 mg·L^{-1} TP in wastewater effluents used for microalgae culture, the permeate had an initial NH_4^+-N concentration of 67.9–98.6 mg·L^{-1} and an initial TP concentration of 9.3–16.6 mg·L^{-1}. Nutrient-rich conditions can lead to difficulty in obtaining entire nutrient removal (Su *et al.* 2012). However, in this work, complete nutrient removal was achieved.

Micronutrient addition to the permeate (A)

Experiments (2) to (5) aimed to determine which chemical played a role in the differences observed between the culture media (A) and (B). The addition of iron (II) sulfate with EDTA as a chelating agent to the permeate permitted the decrease observed in TN removal to be overcome. For an efficient wastewater treatment, where the main goal is to obtain an effluent with low TP and TN concentrations, iron (II) sulfate addition is sufficient. Experiment (5), using the species *Chlorella vulgaris*, showed that the decrease in TN removal observed with permeate culture (A) was not strain specific to *Acutodesmus obliquus* and would probably be observed with most microalgae species. Moreover, it demonstrated clearly that only the addition of Fe to the permeate was obligatory to achieve a complete NH_4^+-N removal.

Micronutrient addition is technically and economically easily realizable at full scale. Based on micronutrient requirement, automated feeding pumps might be applied to the process. From an economic point of view, due to the low concentrations needed for efficient nutrient removal, the addition of iron (II) sulfate and EDTA will cause a maximum additional charge (concentrations of additional iron and EDTA non optimized in this study) of 0.09 €·m$_{permeate}^{-3}$. Considering a biomass production ranging from 2 g·L^{-1} to 4 g·L^{-1}, the additional cost will vary from 0.02 €·kg$_{biomass}^{-1}$ to 0.05 €·kg$_{biomass}^{-1}$. Given microalgae cultivation at full scale costs between 4.16 and 5.96 €·kg$_{biomass}^{-1}$ (Norsker *et al.* 2010), this change will only have a low influence on the cost of the process.

Quality of the final effluent

In this study, high quality effluents regarding macronutrient final concentrations were achieved. This was a preliminary and necessary step before continuous cultures in a full-scale application. The quality of the effluent (B) permitted the fulfillment of the European Commission Directive 98/15/EC for wastewater discharge to sensitive areas that are subject to eutrophication. While a maximum TP

concentration of $1 \text{ mg} \cdot \text{L}^{-1}$ is authorized, the culture (B) reached final concentrations of 0.57, 0.41 and $0.11 \text{ mg} \cdot \text{L}^{-1}$ during (1) and 0.09 and $0.27 \text{ mg} \cdot \text{L}^{-1}$ during (2). With final TN concentrations of 6.54, 4.89 and $5.30 \text{ mg} \cdot \text{L}^{-1}$ during (1) and 7.09 and $9.35 \text{ mg} \cdot \text{L}^{-1}$ during (2), the requirements regarding TN were also fulfilled (maximum TN concentration of $10 \text{ mg} \cdot \text{L}^{-1}$ authorized). Moreover, as pH was kept to neutrality and NH_4^+-N and TP molarity were not similar, it was presumed that TP and NH_4^+-N did not precipitate in the form of struvite (de-Bashan & Bashan 2004). Therefore, it was assumed that nutrient removal from the culture media (A) and (B) was mostly due to nutrient assimilation by the microalgae cells. These results regarding the quality of the final effluent have to be confirmed by full-scale experiments.

Suitability of the permeate for continuous microalgae culture

In this work, permeate enriched with Fe, Mg, Mn, S and EDTA appeared to be a suitable culture medium for the microalgae species *Acutodesmus obliquus*. During experiments (1) and (2), 94% TN removal, 100% NH_4^+-N removal and 98% TP removal were reached and the biomass production was higher than the control culture with commercial fertilizer (C) during the experiment (1). TP was also completely removed from permeate (A) throughout the three batch experiments of (1) and the two batch experiments of (2). However, a significant decrease of TN uptake was observed during the third batch of (1) and the second batch of (2). As iron remedied the decrease, the addition of this micronutrient is both necessary and sufficient to achieve complete TN removal in continuous cultures. The demonstration of the suitability of permeate as a culture medium for continuous microalgae production under these conditions represented the main aim of this study.

Furthermore, in experiment (1), the TN decrease was accompanied by a significant reduction in biomass production. Although this reduction started during the second batch test, the issue could not be clearly identified before the third batch test, where the microalgae growth was almost nonexistent. In contrast, despite the decrease in TN uptake, no reduction in the biomass production was observed during the experiment (2). It was assumed that enough nitrogen and phosphorus were still present in the microalgae cells to ensure an optimal growth during the second batch test of (2). However, starting a third batch test would have assuredly led to a significant reduction in biomass production.

The results of this study will be verified by cultivation of *Acutodesmus obliquus* with permeate in outdoor photobioreactors. If the combination of these two technologies at full scale also permits an effluent respecting the requirements of the European Commission Directive 98/15/EC for wastewater discharge to be obtained, this new decentralized wastewater treatment system could be expanded, especially in remote areas without sewerage.

CONCLUSIONS

The present study concluded that the effluent of a decentralized AnMBR treating fresh domestic wastewater and enriched with micronutrients and EDTA is a suitable culture medium for continuous cultivation of the microalgae species *Acutodesmus obliquus*. Compared to a synthetic culture medium, higher biomass production rates and a faster complete removal of the macronutrients phosphorus and ammonium were reached. In permeate that was not enriched with micronutrients and EDTA, a complete removal of the macronutrient phosphorus was systematically achieved. However, in the course of the successive batch tests, a significant decrease of TN uptake was observed. The addition of iron remedied this issue and ensured an optimal TN removal from the permeate. Given that the use of AnMBRs has expanded in the last decade, this work opens new perspectives for the coupling of wastewater treatment with microalgal biomass production and will be continued at full scale in outdoor photobioreactors.

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