

Article

Opportunities and Challenges from Symbiosis of Agro-Industrial Residue Anaerobic Digestion with Microalgae Cultivation

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Abstract: During the last few years, many studies have tested microalgal systems for nitrogen removal from the digestate. However, most of these studies were carried out using pure culture microalgal strains, which require aseptic conditions and thus cannot be used in full-scale applications. The aim of the present study was to explore opportunities in and challenges of the industrial symbiosis of anaerobic digestion and microalgae cultivation to enhance agro-industrial residue management. Batch tests were carried out to investigate the use of a mixed (open) microalgal consortium to treat the liquid fraction of the digestate for nitrogen removal. Preliminary experiments were performed to choose the carbon supply condition optimizing the growth of the open mixed consortium. In detail, the investigated carbon sources were bicarbonate, under two different carbon to nitrogen ratios, CO₂ via the free surface and CO₂ via air flushing. Further tests were conducted to compare the use of ammoniacal and nitric nitrogen sources. Then, the effectiveness of the liquid fraction of the digestate as nitrogen source was assessed. The highest biomass concentration of 1.6 g L⁻¹ was obtained using CO₂ as carbon source via air flushing as feeding strategy and ammoniacal nitrogen. Biomass production was lower (0.6 g L⁻¹) under the digestate. Nonetheless, due to a probable symbiosis between microalgae and bacteria, a total nitrogen removal of 98.5% was achieved, which was the highest obtained in the present study. Such experimental results address the identification of the steps needed for larger-scale application of combined anaerobic digestion and mixed microalgal systems.

Keywords: anaerobic digestion; circular economy; microalgae technology; mixed consortium; wastewater treatment; waste recovery



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1. Introduction

The recovery of energy from agro-industrial residues constitutes an environment-friendly economic opportunity, leading to improvement in the agri-food waste management [1]. Due to its limited environmental impacts and high potential for energy recovery, anaerobic digestion (AD) is one of the most suitable technologies to reduce the chemical oxygen demand (COD) content of organic wastes [2], including agro-industrial residues. It is conducted by different families of bacteria that—in the absence of oxygen—are able to break down complex organic matter to produce a methane-rich biogas. However, in addition to biogas, the liquid effluent of the process (i.e., digestate) is another abundant product of the AD process. Usually, digestate is separated into solid (10–20% m/m) and liquid (80–90% m/m) fractions [3]. The solid fraction contains less water and is more stable. Therefore, it can be easily transported, stored and used for agricultural or energy production purposes [4]. By contrast, processing the liquid fraction of the digestate is more difficult, and it is becoming a major bottleneck in the development of biogas industries [5]. Currently, the most used management method is direct spread on agricultural lands. However, such a practice has many disadvantages. Most importantly, the liquid fraction of the

digestate is rich in ammoniacal nitrogen, which may cause the eutrophication of nearby water systems [6].

The increasing number of AD plants leading to an oversupply of digestate makes the search for alternative sustainable treatment methods imperative. In this regard, microalgae cultivation may represent one of the most promising solutions for nitrogen removal from the liquid fraction of the digestate [3].

As photosynthetic microorganisms, microalgae are capable of assimilating inorganic carbon and converting it to biomass, which can be successively valorized for the development of valuable biochemicals and biofuels [7].

During microalgae cultivation, nutrients have to be provided besides water, inorganic carbon, and light. Currently, mainly artificial nutrients are used, thus reducing the environmental and economical sustainability of the cultivation process. Nevertheless, it has been estimated that half of the production costs can be avoided by replacing artificial nutrients with waste streams, such as the liquid fraction of the digestate [8].

Many researchers have studied the possibility of removing nitrogen from the digestate within the contextual production of algal biomass. For instance, *Chlorella* strains have been cultivated using digestate of various origin (e.g., animal manure, municipal and industrial wastewater), obtaining good results in terms of both nitrogen removal and biomass production. Moreover, other strains, such as *Desmodemus*, *Scenedesmus*, and *Neochloris*, have been found to be promising. The sustainability of the proposed approach has been studied using life cycle assessment techniques as well. In particular, two recent works by Li et al. demonstrate the environmental advantages of such application [9,10].

The main results, in terms of nitrogen removal and biomass production, achieved in the most recent and interesting studies conducted on this topic are reported in Table 1.

Table 1. Summary of the most relevant studies on ammoniacal nitrogen removal from digestate in microalgal systems.

| Strain | Digestate Source | Pretreatment | Feeding Mode | Biomass Production | Nitrogen Removal Efficiency | Refs. |
|--|--|---|-----------------------------|--|--|-------|
| <i>Chlorella pyrenoidosa</i> | Starch wastewater | Filtration Sterilization Dilution | Batch | 3.01 g/L | TN 91.6% | [11] |
| <i>Chlorella pyrenoidosa</i> | Starch processing wastewater | Precipitation Filtration | Batch | 2.05 g/L | TN 83.1% | [12] |
| <i>Chlorella pyrenoidosa</i> | Municipal wastewater Dairy wastewater | Centrifugation | Batch | 0.6–0.7 g/L | NO ₃ ⁻ 72–89% NH ₄ ⁺ 90–91% | [13] |
| <i>Desmodemus</i> sp. | Pig manure | Filtration Dilution | Batch | 0.412 g/L | TN: 100% | [6] |
| <i>Chlorella vulgaris</i> | Dairy manure | Dilution | Semicontinuous | 0.760 g L ⁻¹ | TN: 93.6% NH ₄ ⁺ : 100% | [14] |
| <i>Chlorella vulgaris</i> | Municipal wastewater | NP | Batch (membrane reactor) | 39 mg L ⁻¹ d ⁻¹ | TN: 56% | [15] |
| <i>Chlorella Phormidium</i> sp. | Dairy wastewater | NR | Batch (biofilm reactor) | 3.1 g m ⁻² d ⁻¹ | TN: 94% | [16] |
| <i>C. vulgaris</i> , <i>Scenedesmus obliquus</i> , <i>Neochloris oleoabundans</i> | NR | Sterilization; Filtration | Batch | 0.01–0.06 gL ⁻¹ d ⁻¹ | TN: 76.0% | [17] |

NP: not performed; NR: not reported.

As can be seen in the table, during the last few years, several studies have been performed on digestate valorization using microalgae. Promising results in terms of both

nitrogen removal and microalgal biomass production have been obtained. Unfortunately, these results are still preliminary. The reported studies were mainly conducted using pure culture microalgal strains, which require carefully controlled conditions and thus can hardly be used in full scale applications.

Conversely, open mixed-culture systems may represent an important step forward in the application of the aforementioned processes. Microalgal consortia, characterized by a strong biodiversity, are more stable and robust compared to pure cultures. Moreover, they own the capacity to absorb light at multiple wavelengths and to assimilate a heterogeneous variety of nutrients [18]. Unfortunately, as depicted in Table 1, the mixed-culture strategy applied to the treatment of digestate is still unexplored.

Therefore, the aim of the present study was the treatment of the liquid fraction of the digestate for nitrogen removal using an open consortium of microalgae. The study was structured in a first experimental phase, consisting in preliminary tests, performed using synthetic media to choose the optimal operating conditions in terms of carbon supply, in addition to assessing the effectiveness of ammonium as a nitrogen source for microalgal growth. Successively, the nitrogen source was replaced with the liquid fraction of the digestate to test the nitrogen removal efficiency and possible inhibiting effects.

2. Materials and Methods

2.1. Digestate and Microalgal Culture

The mixed microalgal consortium was grown from different water samples obtained from fountains and lakes in the Mostra d'Oltremare complex (Naples, Italy). The consortium was enriched in a 200 mL continuously illuminated shaken flask, using Bold's basal medium (BBM) supplied with bicarbonate [19]. After 10 days of incubation, the medium assumed the typical green color of chlorophyll pigments and was used as inoculum for all experiments.

The liquid fraction of the digestate was collected from a full-scale anaerobic digestion plant treating wastewaters of both municipal and industrial origin, located in Mercato San Severino (Salerno, Italy) (GPS data: 40.77884449034634, 14.718874092064496). Before use, the digestate was characterized in terms of: ammonium ($1.13 \pm 0.03 \text{ gNH}_4^+ \text{ L}^{-1}$), nitrates ($41.11 \pm 1 \text{ mgNO}_3^- \text{ L}^{-1}$), phosphates ($73.98 \pm 3 \text{ mgPO}_4^{3-} \text{ L}^{-1}$), sulfates ($32.98 \pm 3 \text{ mgSO}_4^{3-} \text{ L}^{-1}$) and chloride ($260 \pm 5 \text{ mgCl L}^{-1}$).

2.2. Experimental Setup

As carbon represents the most important nutrient for microalgal growth [19], a first set of experiments was performed to choose the carbon supply condition optimizing the growth of the open mixed consortium. To this aim, four experiments were performed using the carbon free BBM, supplied with different carbon sources:

- bicarbonate, added to provide the optimum carbon to nitrogen ratio (C/N) of 100:20;
- bicarbonate, to ensure a carbon-excess condition (C/N = 300:20);
- CO₂ from air, via the free surface;
- CO₂ from air, via air flushing at $300 \text{ mL}_{\text{air}} \text{ min}^{-1}$

A stoichiometric carbon supply of 100:20 was calculated based on the microalgae chemical formula reported by Tuantet et al. [20]. Under the best carbon supply condition, ammonium was tested as nitrogen source. To this end, a modified BBM was used. The modification consisted in replacing nitric nitrogen with ammoniacal nitrogen, using the same nitrogen concentration of 41 mgN L^{-1} . Finally, to assess the effectiveness of the liquid fraction of the digestate as nitrogen source, a further experiment was conducted by adding digestate to a nitrogen-free BBM. To keep the nitrogen concentration equal to those settled in previous tests, the digestate was diluted by a factor of 20. The scheme of the experimental plan and the conditions of all performed experiments are summarized in Figure 1 and Table 2, respectively.

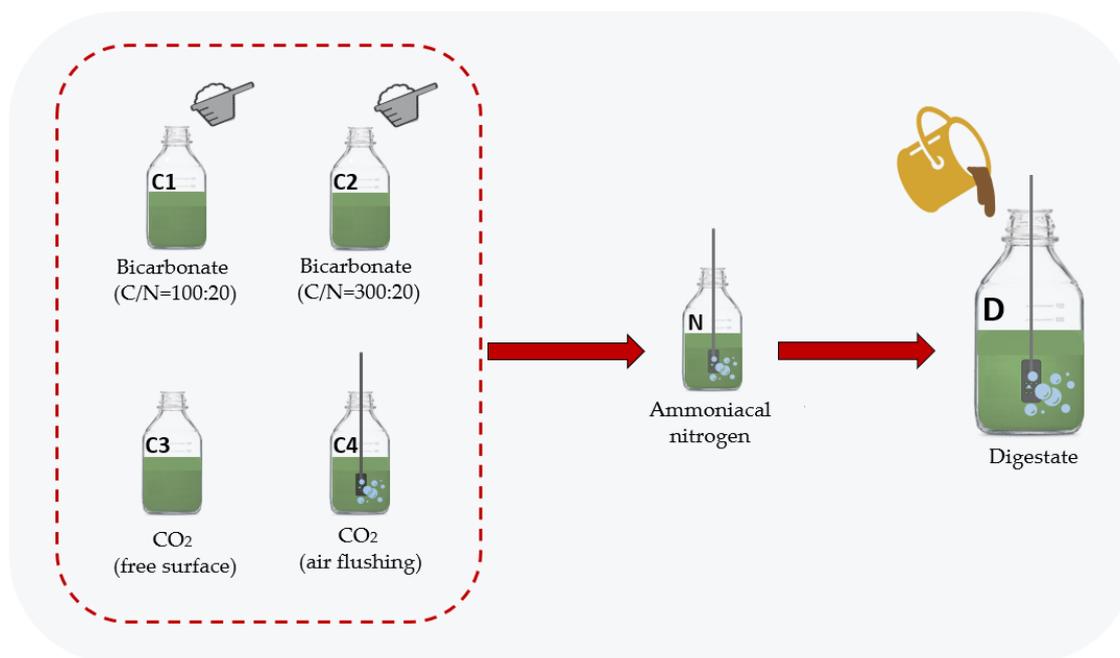


Figure 1. Scheme of the experimental plan.

Table 2. Summary of the experimental conditions.

| Test Code | Carbon Source | Nitrogen Source | C/N Ratio | Nitrogen Concentration |
|-----------|--------------------------------------|--------------------|-----------|---|
| C1 | NaHCO ₃ | NaNO ₃ | 100:20 | 250 mg NaNO ₃ L ⁻¹ 41 mgN L ⁻¹ |
| C2 | NaHCO ₃ | NaNO ₃ | 300:20 | 250 mg NaNO ₃ L ⁻¹ 41 mgN L ⁻¹ |
| C3 | Air (CO ₂), free surface | NaNO ₃ | - | 250 mg NaNO ₃ L ⁻¹ 41 mgN L ⁻¹ |
| C4 | Air (CO ₂), flushing | NaNO ₃ | - | 250 mg NaNO ₃ L ⁻¹ 41 mgN L ⁻¹ |
| N | Air (CO ₂), flushing | NH ₄ Cl | | 146 mg NH ₄ Cl L ⁻¹ 41 mgN L ⁻¹ |
| D | Air (CO ₂), flushing | Digestate | | 15 mL digestate 41 mgN L ⁻¹ |

All experiments were conducted in triplicate, under unsterile conditions. Each condition was tested until the end of the stationary growth phase of the microalgal consortium was achieved. All tests were performed using 0.5 L glass reactors with a working volume of 0.3 L: 30 mL inoculum and 270 mL medium. Reactors were operated in batch mode, under uncontrolled pH, and at room temperature (25 ± 2 °C). As reported in previous studies on photobioreactors, light was continuously provided using LED strips (light intensity: 4000 lux) and agitation was ensured by magnetic stirring at 250 rpm [21,22]. Every two days, 4 mL liquid samples was extracted from reactors to measure biomass growth, nitrogen concentration, pH, total inorganic carbon (TIC) and total organic carbon (TOC).

2.3. Analytical Methods

Biomass growth was indirectly measured via optical density (OD) at 680 nm, referenced to the total suspended solids (TSS) concentration with a standard correlation curve. A WTW PhotoLab spectrophotometer (6600 UV-Vis) was utilized for optical density analysis. Ammoniacal nitrogen was determined using the colorimetric method, in accordance with

the standard methods [23]. Nitrates, nitrates, phosphates, sulfates and chlorides were measured via ionic chromatography using a Metrohm 761 compact ion chromatograph equipped with a Dionex IonPac AS12A 4×200 mm column. TIC and TOC were determined using a Shimadzu (Japan) Total Organic Carbon Analyzer TOC-L. pH was analyzed using an InoLab® (WVR, Italy) Multi 9620 IDS, WTW (Germany) pH meter, equipped with an IDS SenTix® (WVR, Italy) 940, WTW temperature probe, which was used to check the temperature. Light intensity was checked using a Lutron-LX-107 light meter.

All the chemicals used for media preparation and analytical determinations were of high purity. Only double-distilled water was adopted for all analytic procedures. Before each use, glassware was soaked overnight in a concentrated nitric acid bath (2% *v/v*) and rinsed several times using double-distilled water. Before analyses, laboratory equipment was carefully checked using standard solutions.

The following results report the means of data recorded for three replicates, which were performed for each test described in Table 2. Error bars represent the standard deviation. Where not visible, the standard deviation value was lower than 0.07 for the pH and 10 mgL^{-1} for all other measures.

3. Results

3.1. Bicarbonate as Carbon Source

Figure 2 reports tests C1 and C2 results, which were conducted using bicarbonate as carbon source, under different concentrations.

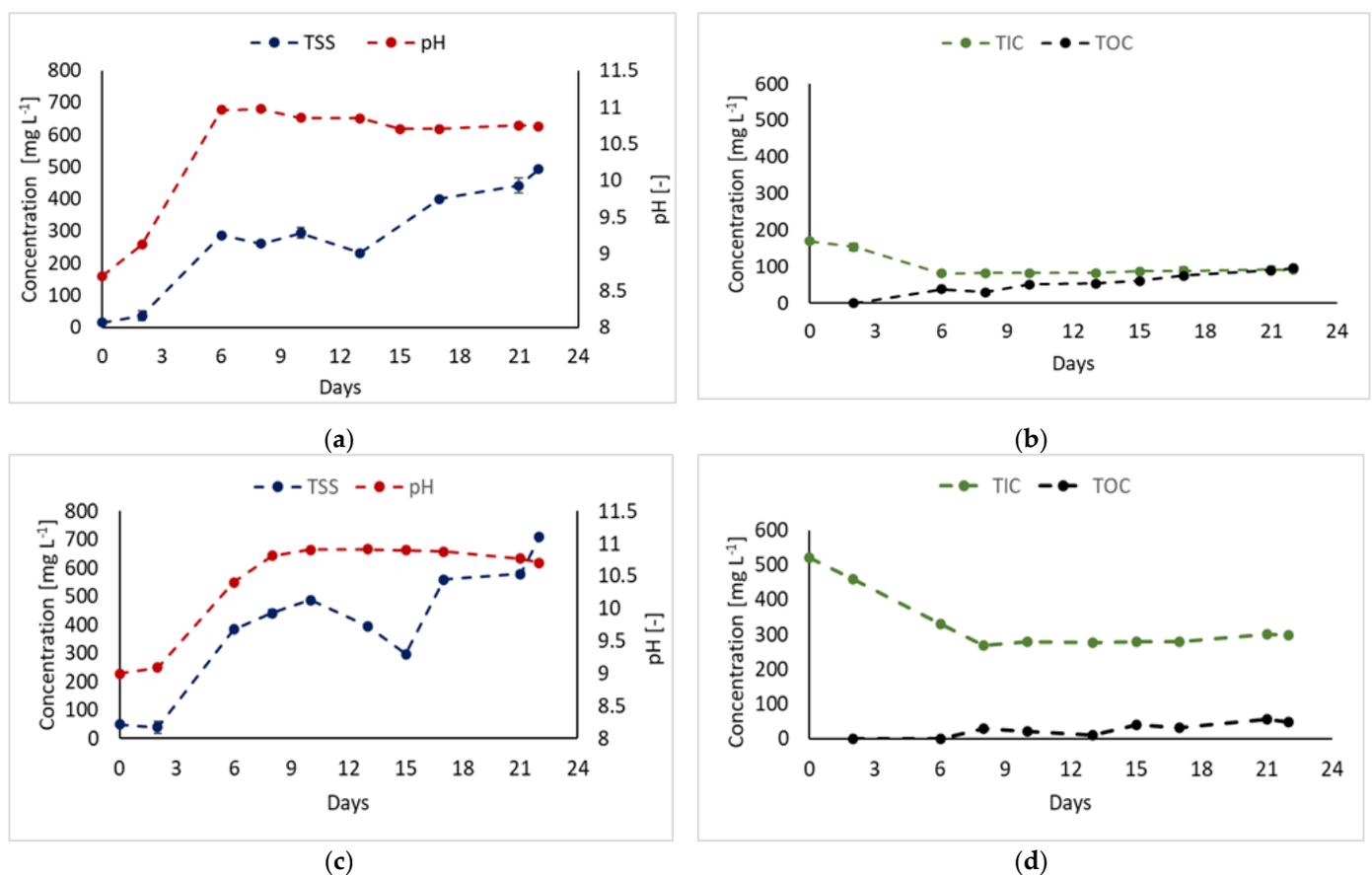


Figure 2. (a,b): Results of experiment C1; (c,d): results of experiment C2. (a,c): Biomass concentration and pH; (b,d) total inorganic carbon (TIC) and total organic carbon (TOC).

Under a C/N of 100:20, it was possible to note a pH increase up to day 6 and a subsequent stabilization around the value of 11. The pH increase was concomitant to the biomass growth. Indeed, the TSS mainly increased during the first 6 days of the process.

After day 6, biomass concentration settled on values of about 300 mg L^{-1} . However, during the last days of the process, a new growth phase occurred and the TSS reached 500 mg L^{-1} . The TIC and TOC trends are in agreement with the biomass curve: the TIC depletion, which indicates bicarbonate consumption, and the TOC increase, which is associated with good photosynthetic activity, are concomitant with the biomass growth. After day 6, it is possible to observe a stabilization of TIC, which kept constant at about 80 mg/L until the end of the test.

Under carbon-excess conditions (test C2), pH and biomass trends are similar to those observed when C:N was 100:20. The biomass reached a value of about 600 mg/l . However, the TIC and TOC values indicate scarce bicarbonate consumption, which presented a residual value of about 300 mgC/L and limited photosynthetic activity, suggesting that the carbon excess could not be consumed, due to the nitrogen limitation.

3.2. CO_2 as Carbon Source

In test C3 (Figure 3a,b), the carbon was provided by the sole free surface contact with atmospheric CO_2 . In this case, the biomass trend is characterized by a continuous growth until day 16, after which it is possible to observe the stationary phase. The final biomass concentration reached a value of about 1000 mg/L . The TIC was almost constant during the whole process since it was continuously provided. Most likely, the IC consumption rate was similar to the loading rate. An increase in the TOC was observed, denoting the photosynthetic activity of the microalgal community.

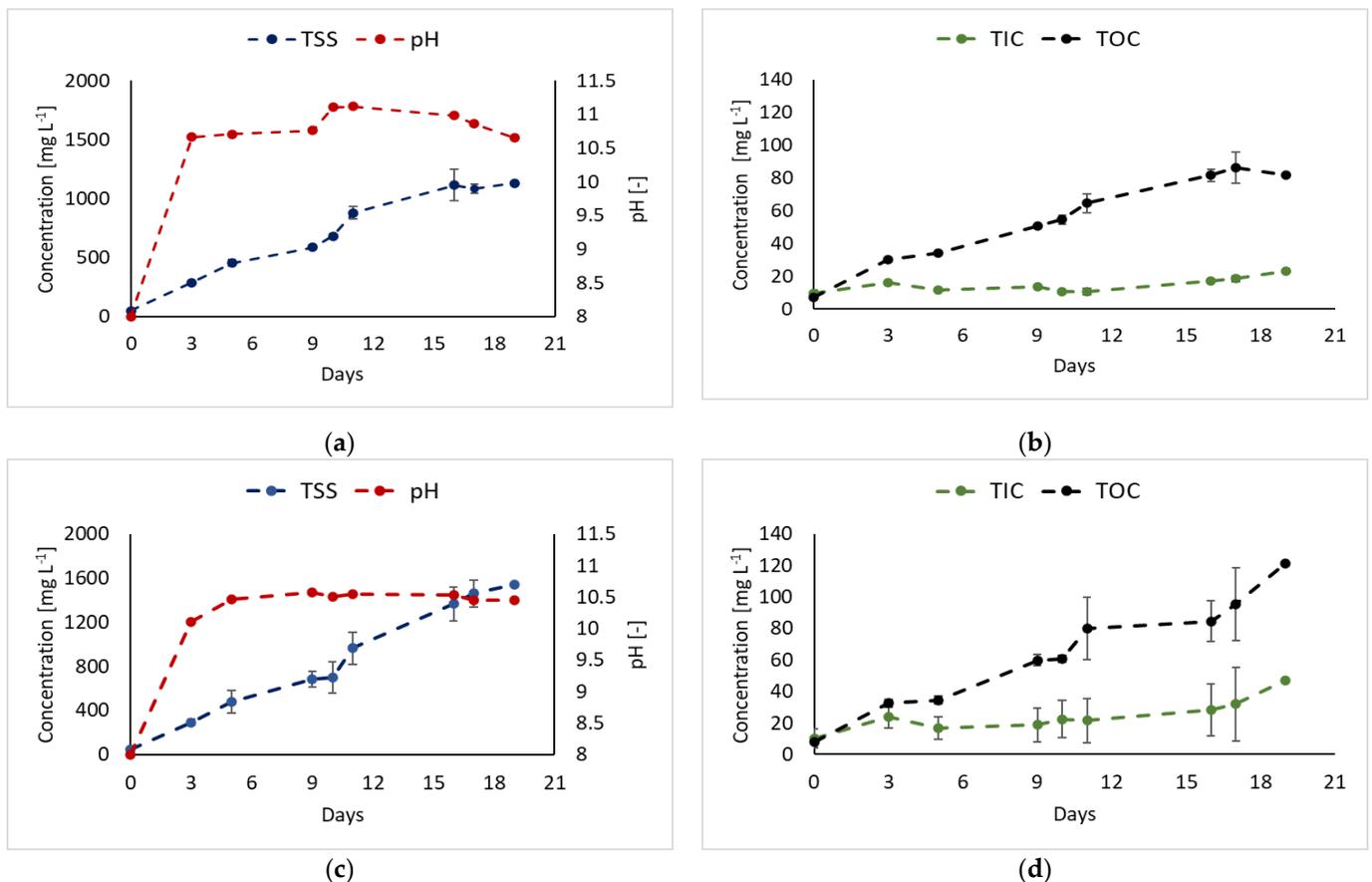


Figure 3. (a,b): Results of experiment C3; (c,d): results of experiment C4. (a,c): Biomass concentration and pH; (b,d) total inorganic carbon (TIC) and total organic carbon (TOC).

When the CO_2 was flushed in the reactor (test C4), the biomass trends were comparable to those observed in test C3. On the other hand, the higher TSS value of 1600 mg/L was reached at the end of the process. The pH kept quite constant at around 10.5. As expected,

such value was lower compared to those obtained in test C3, because of the acidifying effect of the CO₂ flushing. Regarding TIC and TOC, the trends indicate a good photosynthetic activity and carbon consumption, evidenced by the TIC low values and the increase in the TOC values. Comparing the biomass production under the investigated conditions in tests C1, C2, C3 and C4, it is possible to observe that CO₂ was the preferred carbon source and that air flushing was the most suitable carbon feeding mode.

3.3. Nitrogen Source: Ammonium vs. Nitrates

Starting from the results obtained in the first four experiments, CO₂ was chosen as the carbon source and was provided via air flushing. Before feeding the reactors with the digestate, the effectiveness of ammonium as nitrogen source was tested using NH₄Cl (test N). Figure 4 plots the comparison, in terms of nitrogen consumption and biomass growth, between tests C4 and N, which were conducted under the same conditions and with two nitrogen sources.

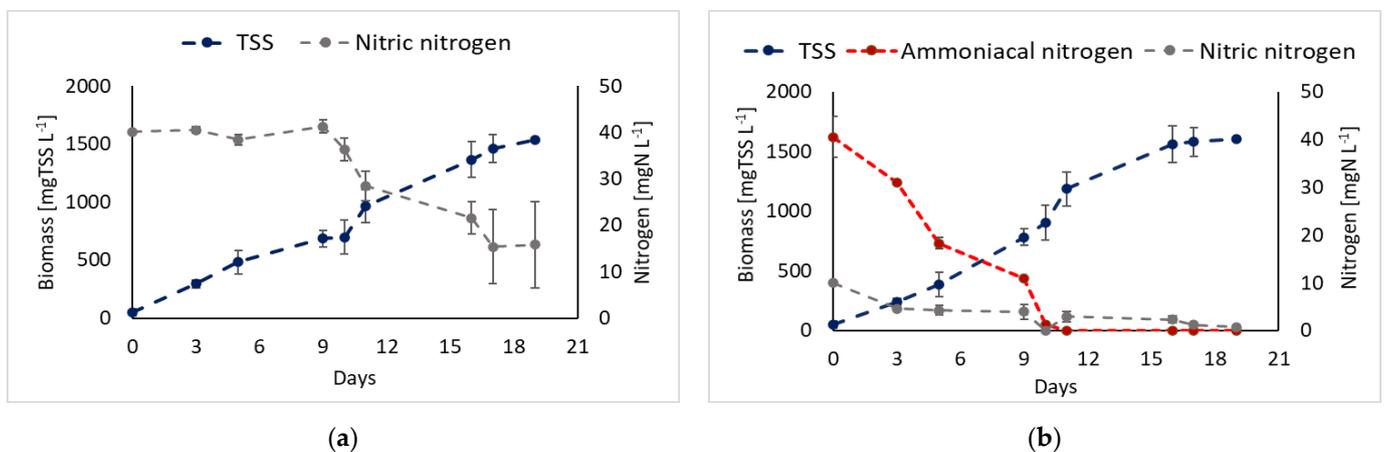


Figure 4. Biomass and nitrogen trend for tests: (a) C4 and (b) N.

When nitrogen was provided as nitrate (test C4), its depletion trend was in accordance with the biomass growth. Indeed, at increasing growth rate, nitrates were consumed faster. However, they were not completely depleted. When ammonium was used, despite the absence of nitrates in the medium, a residual nitric nitrogen concentration was present at the beginning of experiments. Most likely, the nitrate presence was due to a residual concentration in the effluent used as inoculum. During the first days of the process, nitrates concentration kept constant, while ammoniacal nitrogen was consumed for biomass growth. From day 8, the nitrates trend began to decrease. This phenomenon was observed in concomitance with the complete depletion of the ammoniacal nitrogen. In terms of biomass growth, the final TSS concentration was 1539 and 1607 in tests C4 and N, respectively.

3.4. Liquid Fraction of the Digestate as Nitrogen Source

Figure 5 reports results of the experiment conducted using the liquid fraction of the digestate as nitrogen source.

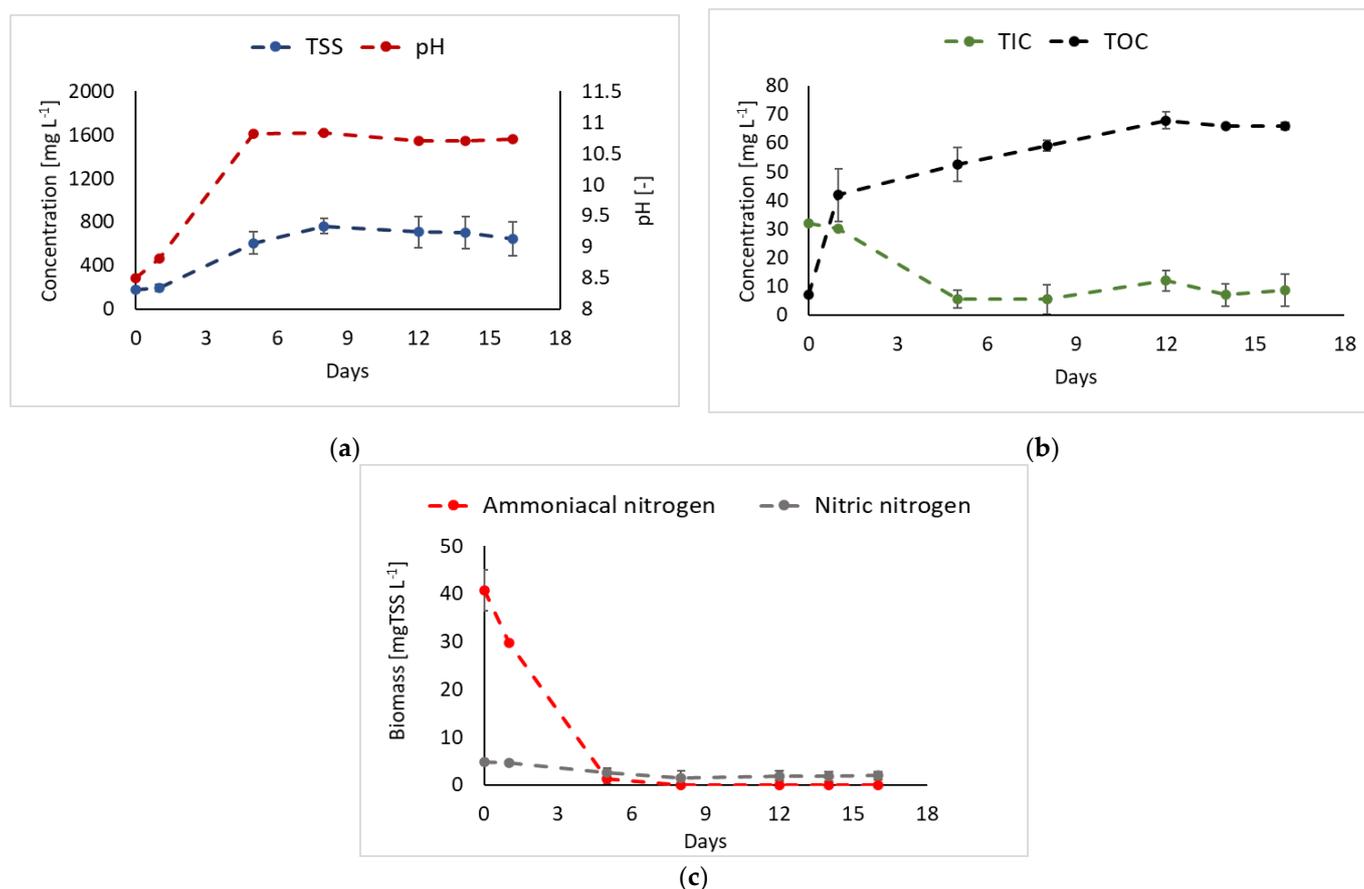


Figure 5. Results of experiment using the diluted digestate as nitrogen source: (a) biomass growth and pH trend, (b) total inorganic carbon (TIC) and total organic carbon (TOC) trends, (c) nitrogen consumption.

Biomass growth occurred from the first day, without any lag period. TSS values kept increasing until day 8. Such increase was contextual to the ammoniacal nitrogen consumption, which was completely depleted after 5 days. During the following days, a stationary phase, characterized by a final TSS values of 950 mg/L, was observed. During the last few days of monitoring, the TSS values showed a decreasing trend, which indicates the lack of nutrients, in accordance with the nitrogen depletion curves. Concerning the nitrates trend, it is possible to observe a first phase characterized by a constant concentration and a second phase, after ammonium depletion, characterized by nitrate-decreasing trends. Concerning TIC and TOC trends, an almost immediate increase in the TOC and a simultaneous decrease in the TIC were detected. Such trends, indicating the carbon source consumption and the photosynthetic activity, are in agreement with the biomass growth curve. As previously observed, TIC and TOC were almost constant when the biomass did not grow anymore.

Table 3 summarizes the most relevant results from tests C4, N and D, which were all conducted under the same carbon feeding strategy and nitrogen concentration, using synthetic nitric nitrogen, synthetic ammoniacal nitrogen and digestate.

Table 3. Summary of the main results in terms of nitrogen removal and algal biomass production.

| Test Code (N-Source) | Total Nitrogen Removal [%] | Ammoniacal Nitrogen Removal [%] | Nitric Nitrogen Removal [%] | Biomass Production [gTSS L ⁻¹] |
|-------------------------|----------------------------|---------------------------------|-----------------------------|--|
| C4 (NaNO ₃) | 62 ± 20 | - | 62 ± 20 | 1.53 ± 0.01 |
| N (NH ₄ Cl) | 96 ± 1 | 100 ± 0.00 | 60 ± 1 | 1.61 ± 0.01 |
| D (Diegstate) | 98.5 ± 1 | 100 ± 0.00 | 93 ± 1 | 0.65 ± 0.04 |

Using synthetic nitrogen in the form of nitrates, nitrogen removal was not complete. Nevertheless, the mixed consortium was able to completely remove ammoniacal nitrogen in both tests N and D. The use of synthetic media promoted biomass growth, which seemed limited in the presence of digestate.

4. Discussion

Differently from the majority of literature studies conducted using pure strains, in this study, an open mixed consortium was cultivated and successively used in different experiments, to study both the carbon source and feeding strategy to enhance the growth of the consortium while removing nitrogen from the culture media.

From the results, it is possible to state that even though the biomass growth was not affected by the C/N ratio, the photosynthetic activity was enhanced by the stoichiometric C/N ratio of 100 compared to a carbon-excess condition (C/N = 300). Under carbon excess, the TIC accumulation occurred within the photobioreactor, suggesting that nitrogen can lead the kinetic reactions acting as the limiting agent. The enhanced biomass production obtained by providing carbon via the sole contact with atmospheric carbon dioxide indicates that bicarbonate was not the preferred inorganic carbon source of the microalgal consortium under investigation.

This result is not in agreement with previous findings reporting that under nutrient-sufficient conditions with NaHCO_3 as carbon source, biomass growth almost doubled compared to the cultivation under aeration alone [24]. Nonetheless, another study indicated that the growth of a mixed culture dominated by *Chlorella vulgaris* and *Scenedesmus armatus* was inhibited by bicarbonate [25]. These contrasting results may be due to the fact that optimal carbon inputs strongly vary depending on the microalgal strain.

The metabolic mechanism of NaHCO_3 utilization by microalgae differs from CO_2 uptake. The bicarbonate transporters are embedded in the chloroplast envelope and plasma membrane in microalgal cells. Bicarbonate ions (HCO_3^-) are converted to CO_2 by carbonic anhydrase in the periplasmic space, which is then absorbed and utilized by microalgal cells [26]. The correlation between pH and TSS in C1 and C2 tests suggest that the high pH value may have caused the biomass growth limitation. Indeed, based on the pH value, carbon can be available in different forms. In particular, when $8 < \text{pH} < 10$, the carbon is present in the form of carbonate ions, whilst for $\text{pH} > 10$ it is available in the form of bicarbonate ions. According to previous studies, carbonate ions are the most bioavailable form of carbon for microalgae, which are therefore favored by pH values lower than 10 [27].

The highest biomass concentration was obtained using CO_2 as carbon source and air flushing as feeding strategy. This is in good agreement with previous studies conducted on pure cultures, confirming the effectiveness of the air flushing technique [28]. In addition, according to previous findings, the use of CO_2 as carbon source can increase biomass production and photosynthetic activity, due to the upregulation of protein activities related to the TCA cycle and photosynthesis, such as phosphoenolpyruvate carboxylase, pyruvate carboxylase and ferredoxin [29]. Such previous findings, together with the results reported in the present work, are encouraging for mixed-consortia future applications on CO_2 capture and utilization technologies [30]. Indeed, the utilization of CO_2 from industrial gaseous streams could be also an effective method to reduce carbon emissions from power plants [31].

Even though the carbon source determined a more marked effect on biomass growth compared to the nitrogen source, the use of ammoniacal nitrogen enhanced the final TSS value. Moreover, the nitrogen removal process was much more efficient in the presence of ammoniacal nitrogen compared to nitric nitrogen.

Such results are consistent with a previous study conducted on the accumulation of microalgal lipids using pure cultures. The authors compared the use of ammonium and nitrates and found that the enhanced lipids productivity under ammonium feeding conditions was mainly due to the biomass increase [32]. The biomass increase can be attributed to the higher electron availability in the presence of ammoniacal nitrogen. Indeed,

before being assimilated, nitrate has to be reduced to nitrite and then to ammonium by reducing enzymes [33]. Therefore, because of its oxidized form, nitrate is energetically more difficult to be assimilated compared to ammonium [34].

As nitrogen is mostly present as ammonia in the digestate, further experiments were carried out using diluted digestate as nitrogen source, but in this case, microalgal growth was limited. This phenomenon was already observed in previous studies conducted using pure cultures and it is ascribable to the shading effect exerted by the dark color of the digestate. Nonetheless, the achieved TSS concentration is similar to those reported for pure cultures [6,13]. Moreover, nitrogen removal was even higher compared to the control test, performed using NH_4Cl . It is likely that the ammoniacal nitrogen consumption was due to a synergistic effect of microalgae and nitrogen-consuming bacteria, as reported in previous research [35]. Nonetheless, the effect of microalgae on nitrogen removal can be confirmed by the correlation of the nitrogen consumption curve and the detected microalgal growth. Moreover, as microalgae need nitrogen for biomass generation, the detected microalgal biomass increase cannot be separated from the nitrogen uptake: Hypothesizing that the active biomass (not including intracellular compounds, such as carbohydrates and lipids) was 60% of the total TSS and using the microalgae biomass formula ($\text{C}_5\text{H}_{8.9}\text{O}_{1.8}\text{N}_{0.6}$) [36], it emerges that 69% of the consumed nitrogen was used for active biomass generation. The remaining part could be devoted to nitrogen-containing compounds, such as proteins. In addition, as previously reported, bacteria in symbiosis with microalgae could also have played a role in removing nitrogen [37].

Comparing the present study with previous papers reported in Table 1 and with a recent review by Tawfick et al. [38], it emerges that the present results are similar to or even better than those reached using pure cultures. Regarding the use of mixed cultures, the present results are similar to those reached by Ermis et al. [39], who studied a mixed (open) microalgal consortium using the liquid fraction of the digestate from an AD plant treating the organic fraction of the municipal solid waste, cattle and chicken manure and the expired market waste and leaching water from waste collection vehicles. The achieved results in terms of biomass production and nitrogen removal ranged between 1 g L^{-1} to 1.36 g L^{-1} and 78% to 88%, respectively.

Overall, experimental outcomes show that open mixed consortia are effective for the nitrogen removal process from the digestate, with the additional advantage that do not require aseptic conditions, as the contamination can even represent a value. The use of this kind of consortium may thus pave the way for novel industrial symbiosis schemes combining anaerobic digestion and microalgal cultivation, in which the former can provide the sustainable valorization of agro-industrial residues via the generation of energy and the latter can ensure the environmentally friendly treatment of anaerobic digestion emissions via carbon dioxide and ammonia nitrogen upcycling for the generation of a microalgal biomass that can be further valorized. In real applications, it is possible to perform a settlement pretreatment process to reduce the turbidity of the wastewater, which in turn improves light penetration and microalgal biomass growth [9]. Moreover, in order to improve the environmental feasibility and economy of a real scale process, it is paramount that natural light be used instead of artificial sources. Future research on mixed open consortia testing the mentioned possibilities are required. In addition, a key challenge in mixed consortia applications is to ensure microalgae domination over bacteria. Consequently, successful full-scale cultivation of microalgae (particularly on digestate) requires close monitoring and regulation of biotic and abiotic conditions.

5. Conclusions

The present paper shows that the application of mixed microalgal consortia is an effective solution to remediate the excess of nitrogen from the liquid fraction of the digestate. Nonetheless, culture conditions, such as the carbon source and the feeding strategy, should be carefully adjusted to promote algal biomass growth. Such results are promising; however, further studies are required for scaling up the process. Future research aimed at real-scale

applications should proceed in the direction proposed in this work by testing further digestate-dilution factors, investigating other biotic and abiotic key factors, and scaling up the combined process for environmental and techno-economic feasibility analysis for its reliable implementation.

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