A novel option for reducing the optical density of liquid digestate to achieve a more productive microalgal culturing

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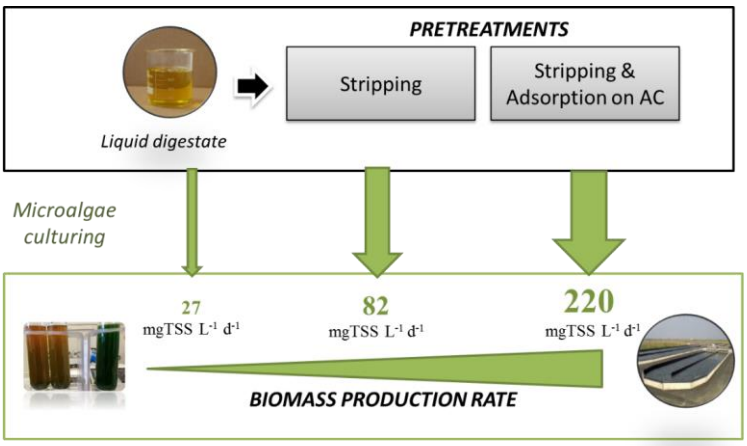
Highlights

- Adsorption improves the optical density of the liquid fraction of agro-digestate
- Microalgae can grow on both raw and pretreated liquid fraction of agro-digestate
- Pretreatments by stripping and adsorption improve algal productivity
- Pretreatments allow reducing the HRT and footprint of algal culturing
- Pretreatment by adsorption limits the occurrence of nitrification in algal reactors

Graphical abstract


PRETREATMENTS
Stripping
Stripping & Adsorption on AC
Liquid digestate
Microalgae culturing
27 mgTSS L-1 d-1
82 mgTSS L-1 d-1
220 mgTSS L-1 d-1
BIOMASS PRODUCTION RATE

Abstract

The liquid fraction of digestate produced by agricultural biogas plants is rich in macro and micronutrients that are valuable for the culturing of microalgae. Nonetheless, the high ammonium concentration may cause toxicity and the high optical density may reduce light penetration, negatively affecting the biomass production rate. Dilution with fresh water has been frequently suggested as a mean for improving the digestate characteristics in view of microalgal culturing. In this paper, the feasibility of culturing microalgae on undiluted raw digestate or on digestate after pretreatment by stripping and adsorption was investigated.

First, adsorption tests were performed using commercial activated carbon from wood in order to identify appropriate conditions for optical density (OD) reduction. Up to 88% reduction was obtained by dosing 40 g/L after 24 h of contact time. Then, culturing tests were performed on a microalgal inoculum including mainly *Chlorella* spp. and *Scenedesmus* spp. under controlled temperature and light conditions during 6-14 weeks. Raw, stripped, and stripped and adsorbed digestate samples were tested. The biomass production rate increased from 27±13 mgTSS L^{-1} d^{-1} on raw digestate, to 82±18 mgTSS L^{-1} d^{-1} by using stripped digestate, and to 220±78 mgTSS L^{-1} d^{-1} by using the stripped and adsorbed digestate. Moreover, nitrification was constantly suppressed when using the stripped and adsorbed digestate, while relevant nitrite built-up was observed when using raw and stripped digestate. These results suggest that microalgae are able to grow on the raw digestate, provided that long hydraulic retention times are applied. A much faster growth (up to 10 times) can be obtained by pretreating the liquid fraction of digestate by stripping and adsorption, which may be an effective means of improving the areal productivity of microalgal culturing on digestates.

Keywords: Microalgae; digestate; optical density; stripping; adsorption.

1. Introduction

In agricultural areas, biogas technology is extensively used for reducing organic matter load from livestock wastewater, through the transformation of organic carbon into biogas, which is in its turn a renewable biofuel [1]. Anaerobic digestion (AD) improves the environmental sustainability of animal breeding and full-scale

installations (biogas plants) have spread rapidly in Europe. As a result of this rapid development, huge amounts of digestate are produced and generally separated into liquid/solid fractions at farm scale. The liquid digestate is rich in nitrogen and potassium and is normally spread into fields as fertilizer, whereas the solid phase, rich in phosphorous and stabilized carbon, is generally used as soil amendment. Nonetheless, due to restrictions imposed by to the European Nitrate directive (91/676/CEE), but also to economic and environmental issues, its conventional valorization route as soil amendment and/or fertilizer does not always fit the local context of intensive livestock areas [2]. Indeed, liquid digestate spreading may cause significant environmental problems such as water contamination and eutrophication [3]. This aspect has prompted attention toward technical solutions to reduce the nitrogen load of digestate, either by chemical-physical processes such as stripping, evaporation, membrane filtration [4] or by advanced biological processes, such as the anammox process [5]. Recent studies have focused on the possibility to use liquid digestate as a nutrient source for microalgae growth [2, 6]. According to Xia and Murphy [3], the land requirement for microalgal cultivation is estimated as 3% of traditional direct land application of digestate. Furthermore, in a concept of circular economy, microalgae grown on digestate can be sent back to the anaerobic digester as co-digesting feedstock or used on soil as a slow-release fertilizer [2], [7] and [8].

However, two major drawbacks of using the liquid fraction of digestate as a substrate for microalgae growth are related to its high nitrogen content and turbidity level [3] and [9]. Indeed, the liquid fraction of digestate is often characterized by: a high concentration of total suspended solids, causing an inherently high turbidity and reducing light transmission, and of ammonia (>100 $mgN \cdot L^{-1}$), which could potentially be toxic to microalgae [10]. Thus, pre-treatments have been often adopted to remove or dilute the undesirable compounds from supernatants. As concerns turbidity, the most common pre-treatments include solid/liquid separation [11], filtration [12] and dilution [13] which also helps in lowering the ammonium concentration. Another novel and promising option is the use of activated carbon (AC) for reducing the turbidity level of liquid digestate, and thus facilitating microalgae growth. The use of activated carbon has attracted attention due to its high capacity of adsorbing various kinds of components [14], [15] and [16]. Furthermore, this activated carbon could be produced from renewable biomasses like lignocellulosic biomasses (*i.e.* wood) through a two steps process: char production by pyrolysis, followed by steam or chemical/thermal activation [17] and [18]. The use of AC for turbidity reduction has been largely investigated for wastewater or seawater treatment with good results [19],

[20] and [21]. Nonetheless, until now, no studies reported the use of AC to reduce the turbidity of liquid digestate in view of microalgae culturing.

The main objective of this study was to evaluate the feasibility of growing microalgae on digestate without addition of fresh water for dilution. To this aim, undiluted (raw) digestate was first tested as microalgal growth medium. Then, pretreatments (ammonia stripping and a combination of stripping and adsorption on AC) were applied to digestate to reduce ammonium concentration, turbidity, and optical density, thus facilitating microalgae growth. First, the effect of the AC concentration (in the range 1 to 40 $g \cdot L^{-1}$) and contact time (in the range 0 to 1440 min) on optical density reduction was investigated. Then, microalgae culturing tests were conducted on raw and pretreated liquid digestate.

2. Materials and methods

2.1 Liquid digestate characterization

The liquid fraction of digestate came from a piggery farm in Northern Italy, breeding up to 20,000 pigs. At the hosting farm, a full-scale wastewater treatment plant (WWTP) and a full-scale anaerobic digester followed by a solid/liquid separation were in operation. The anaerobic digester was fed on the solid fraction of piggery manure (after floatation), poultry manure, maize silage and cheese whey (more details in Scaglione et al. 2015 [5]). The liquid fraction of digestate was collected after solid/liquid separation performed by centrifugation and is here after referred to as DIG_L.

2.2 Adsorption tests

Adsorption tests were carried out by using a commercial activated carbon (AC) from wood (Norit® CA1, Sigma Aldrich, Saint Louis, USA). Tests were carried out at room temperature (20°C), under static conditions, by varying the solid loading of AC from 1 to 40 $g \cdot L^{-1}$, and the adsorption time from 0 to 1440 min. After adsorption, samples were centrifuged at 12200 rpm for 2 min (MiniSpin®, Eppendorf, Hamburg, Germany) and the supernatant was separated and used for optical density analysis.

Optical density (OD) at 680 nm was used to quantify the clarifying effect of the adsorption process. Indeed, the primary pigment involved in photosynthesis is chlorophyll a, which has strong absorption bands in the regions 400–450 and 650–700 nm [22]. According to preliminary tests (data not shown), the mixed microalgal community used in the algal growth tests showed two maxima in the adsorption spectrum at 420 and 680 nm. A strong correlation (data not shown) was observed between OD at 680 and at 420 nm measured on samples of adsorbed DIG_L, suggesting that just one OD determination could be made and used as the reference parameter to assess the clarifying effect of the AC adsorption process. Therefore, the clarification effect of the adsorption process was assessed by comparing the OD level at 680 nm of the original DIG_L ($OD_{digestate}$) to that of the adsorbed digestate ($OD_{adsorbed\ digestate}$), as follows:

$$\text{OD reduction (\%)} = (OD_{digestate} - OD_{adsorbed\ digestate}) / (OD_{digestate}) \quad \text{Eq. 1}$$

2.3 Microalgal culturing tests

Continuous algal culturing tests were performed in 150 mL glass test tubes (4.5 cm diameter, 20 cm height). Light was provided by 6 fluorescent lamps (FLUORA model, OSRAM, Munich, Germany), 18 W each one, with 12 h dark/light periods. The PAR value measured at the test tube location was 130 μmol m^{-2} s^{-1}. Air was flushed from the bottom of each tube through a fine bubble diffuser to maintain well mixed conditions. Temperature remained around 20±2°C.

2.3.1 Tests on raw liquid digestate

Each test tube was prepared by mixing a concentrated microalgal inoculum (10 mL) with 15 mL of DIG_L and 125 mL of tap water. The initial condition was selected in order to start from a non-inhibiting ammonium concentration and to ensure sufficient algal inoculum to ensure a rapid colonization. Specifically, the following conditions were set in each test tube: 160 $mgN \cdot L^{-1}$ of ammoniacal nitrogen concentration and an OD of 0.5. As for the algal inoculum, a mixed microalgal community dominated *by Chlorella* spp. and *Scenedesmus* spp., grown on the liquid digestate from a digester fed on waste activated sludge (Carimate (CO) wastewater Treatment Plant) was used [23]. The algal biomass was centrifuged (10 minutes, 3000 rpm) and a 10 mL aliquot, containing 0.255 g of dry solids, was added to each tube. The starting optical densities of the tubes were 0.50.

Every 1 or 2 weeks, a sample (10 out of 150 mL) of the algal suspension was withdrawn and substituted by an equivalent volume of DIG_L. The frequency of feeding was adjusted in order to achieve an almost complete depletion of the ammonium concentration before each feeding, thus avoiding too high ammonium concentration after each feeding event, which remained below 160 $mgN \cdot L^{-1}$. Tests were performed in triplicate. The concentration of N forms, chemical oxygen demand (COD), phosphorus, total and volatile suspended solids (TSS and VSS), pH, temperature, OD at 680 nm and algal counts were assessed on samples of the algal suspension withdrawn from the test tubes.

2.3.2 Tests on stripped and adsorbed liquid digestate

During these experiments, microalgae were cultured on two types of pretreated liquid digestates. The first one was pretreated by a stripping phase and will be referred to as DIG_S; the second one was prepared by further treating the stripped digestate with AC in order to reduce the OD (DIG_S&A). The stripped digestate was prepared using an air compressor providing 0.6-1.2 vvm through a fine bubble diffuser. No alkaline chemical was used since alkaline pH (around 9-9.5) was naturally achieved during air bubbling. The stripping process lasted from 5 to 7 days at 20±2°C.

The DIG_S&A was prepared by treating the stripped digestate with activated carbon. Following the results of the adsorption tests, a solid load of 40 g AC L^{-1} and an adsorption time of 10 min were chosen. The adsorption process was carried out under static conditions at ambient temperature (20 °C). Then, the activated carbon was separated by centrifuging (3000 rpm for 5 minutes).

Three batches of DIG_S and DIG_S&A were prepared to be used to feed the microalgal cultures. Each one was prepared from a freshly collected DIG_L which was first stripped and then adsorbed on AC. Each batch of DIG_S and DIG_S&A was then stored at 4°C and used for microalgal feeding for no more than 2 weeks. Optical density at 680 nm, ammonium, nitrate, nitrite, soluble COD and phosphate concentrations were assessed for each batch of DIG_S and DIG_S&A.

Culturing tests were performed in duplicate on both DIG_S and DIG_S&A. The algal suspension from the first set of experiments (using DIG_L) was used. To this purpose, replicates from the first tests were mixed and centrifuge (10 minutes, 3,000 rpm). The algal pellet was used to inoculate the glass tubes. Specifically, 10 mL of algal were added in each tube; then, digestate, either DIG_S or DIG_S&A, was dosed to achieve the final working volume of 150 mL. The obtained optical density was between 1.8 and 2.3. Every 2 – 5 days, a fraction (70 mL) of the algal suspension was withdrawn and substituted by either DIG_S or DIG_S&A. The feeding frequency was the same for all the test tubes and defined in order to allow for the almost complete depletion of ammonium in those test tubes fed on DIG_S&A. The same feeding frequency was also adopted for test tubes fed on DIG_S.

The monitoring plan was similar to the one described previously in section 2.3.1.

2.5 Analytical determinations

Solid content (TSS and VSS) Biochemical Oxygen Demand at 5 and 20 days (BOD_5, BOD_{20}) were analyzed according to Standard Methods (APHA, 2005). BOD_5, BOD_{20} were measured on 0.45 µm filtered samples to assess the BOD soluble fraction ($BOD_{5\ sol}$ and $BOD_{20\ sol}$). Ammonia, nitrite and nitrate nitrogen, phosphate and soluble COD (COD_{sol}) were measured using spectrophotometric test kits (Hach-Lange, Germany, LCK 303, LCK 340, LCK 342, LCK 348 and LCK1414, respectively), on 0.45 µm filtered samples. pH and conductivity were measured by a portable meter (XS PC 510 Eutech Instruments, USA). Optical density was measured by a DR 3900 Hach Lange (Germany), spectrophotometer at 680 nm and by using a cuvette with a 1 cm path length.

Microalgae were counted using a Hemocytometer, (Marienfeld, Germany) and a microscope (B 350, Optika, Italy). A sample of 1 mL of microalgal suspension was withdrawn from each vial and conveniently diluted. Then, 0.1 mL of the sample was injected into the hemocytometer chamber. The number of *Scenedesmus* and *Chlorella* algal cells were distinguished according to their morphological characteristics and counted, and the final estimated cell number was obtained from the mean of 9 square (1 mm^2) readings. In case rotifers and nematodes were spotted during the microscopic observation, their presence and qualitative abundance was recorded.

2.6 Statistical analysis

Experiments were performed in duplicate or triplicate, results are expressed as mean ± standard error. The statistical analyses were conducted using the R Project software (R core Team 2015). The T test for paired data was performed to detect differences between daily biomass production and biomass concentration (in term of optical density), when using stripped digestate (DIG_S) and stripped digestate with activated carbon (DIG_S&A) as feed. p-values < 0.05 were deemed to be statistically significant.

3. Results

3.1 Chemical composition of liquid digestate

Three samples of the liquid fraction of digestate were collected from the farm within a 6 months period. The average chemical composition is reported in Table 1. One can see that liquid digestate has highly variable

characteristics, which can be chiefly explained by seasonal variations in the piggery waste production, and in the digested co-substrates, as already shown in Scaglione et al. [5]. For example, the sample of DIG_L (17/7/2015) is characterized by low pH and high COD, probably due to a temporary of overloading of the digester causing an incomplete digestion process.

Liquid digestate had a high OD due to the high concentration of suspended and dissolved solids which limit light penetration and therefore photosynthesis and microalgae growth, as already pointed out in previous researches [6], [24] and [25]. The concentrations of nutrients and COD, their values fall within typical literature intervals [3]. It is worth noting that the liquid digestate had an ammonia concentration between 0.6 and 1.7 g $N \cdot L^{-1}$; high level of ammonia have previously been suggested to be potentially toxic to microalgae [10], while low P concentrations could have limiting effects on microalgal growth [26]. As for the N:P ratio, it varied within a large interval (48±32 g N g P^{-1}). Such values are well above the theoretically optimum value of 10-16 mol N mol P^{-1} (corresponding to 4.5-7.2 g N g P^{-1}) suggested in literature [27]. However, the N:P ratio is within the range of other digestates previously tested for their capacity to sustain microalgal growth. Indeed, Marcilhac et al. [28] observed that the N:P ratio of digestate samples ranged from 38 to 135 g N g P^{-1} and variations depended on the biogas plant and on time at a given plant.

In conclusion, in order to use DIG_L as substrate for microalgae culturing, either dilution or pretreatment may be useful to improve light penetration and to reduce ammonia concentration.

Table 1. Chemical composition of liquid digestate (DIG_L) measured on three samples at different sampling time, and average value (mean±std).

Parameter	Sampling date			mean± std
	18/06/2015	17/07/2015	10/01/2016	
TSS (g L^{-1})	0.79	3.28	0.2	1.4±1.6
VSS (g L^{-1})	0.67	2.93	n.a.	1.8±1.6
pH	8.19	6.75	8.08	7.7±0.8

Commentato [V1]: O standard error?

Commentato [V2]: O standard error?

$N\text{-}NH_4$ (g L^{-1})	1.3	0.6	1.7	1.2±0.5
$P\text{-}PO_4$ (mg L^{-1})	26	41	22	30±10
Optical Density (-)	1.02	1.88	1.039	1.3±0.5
COD_{sol} (g L^{-1})	1.9	8	2	4.0±6
Total COD (g L^{-1})	3.4	9.1	n.a.	6.3±4
BOD_5 sol (g L^{-1})	0.56	0.65	n.a.	0.6±0.1
BOD_{20} sol (g L^{-1})	0.9	1.12	n.a.	1.0±0.2
N:P	50	15	79	48±32
COD:N	1.5	12.6	0.9	5.0±6.6

3.2 Effect of AC adsorption on the optical density of liquid digestate

Figure 1 shows the OD reduction of liquid digestate at different AC dosages (1 to 40 g L^{-1}) and adsorption times (0 to 30 min). As observed, the efficiency of the optical density reduction increased as the AC concentration increased. The best condition for OD reduction was obtained at a AC dosage of 40 g L^{-1} and at an adsorption time of 10 min, corresponding to an OD reduction of 88%. Indeed, after 10 min, the OD reduction improved slightly but not significantly. A picture comparing the raw and adsorbed digestate obtained by applying the previously identified adsorption conditions can be found in Figure 2. Additional correlations between the AC dose and the OD reduction, as well as the trend of the OD during time in longer adsorption tests are available in Appendix A.

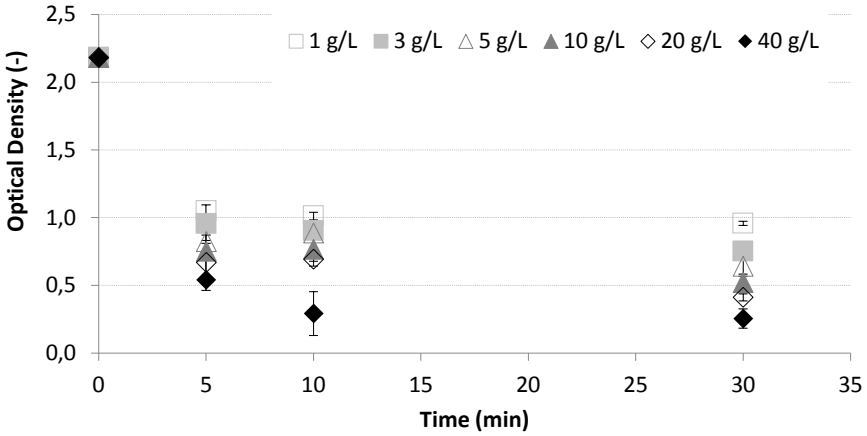


Figure 1. Optical density reduction (%) during the first 30 min in adsorption tests with different AC doses (from 1 to 40 g L^{-1}).

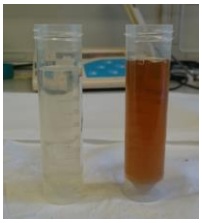


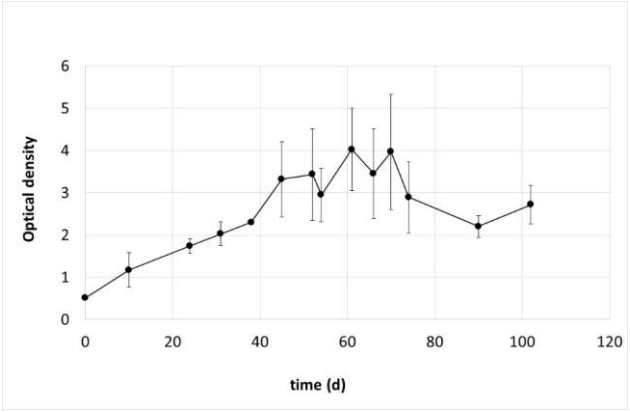
Figure 2. Visual evidence of the OD reduction upon AC adsorption. DIG_L before (right vial) and after (left vial) the adsorption step.

3.3 Microalgal culturing tests

3.3.1 Growth on raw liquid digestate

Results of the first tests of algal growth on DIG_L with a 1,730 mgNH_4-N L^{-1} concentration are reported in Figure 3. Microalgal concentration increased over time up to 3-4 times the initial value. Above such value, light penetration was probably insufficient to sustain further algal growth. Between day 41 and 80, a poor repeatability was observed associated to a significantly higher biomass concentration in one of the triplicate test tubes during that same period. From TSS data, the cumulated biomass production (CBP, mg TSS) was computed and compared to the cumulated volume of DIG_L fed to each test tube (Figure 3). Results show that the amount of algal biomass produced is proportional to the volume of DIG_L fed during each fed-batch cycle. The highest values of specific biomass production rate were observed between day 52 and 74, during which the average hydraulic retention time (HRT) was 55 d and corresponded to 27±13 mg TSS L^{-1} d^{-1}.

a)



b)

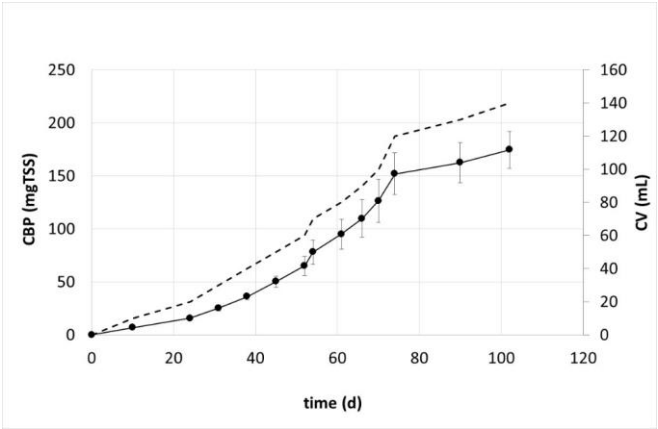


Figure 3. Results of the algal culturing test on raw DIG_L: time trend of (a) optical density, (b) cumulated biomass production (CBP, rounds) and cumulated treated DIG_L volume (CV, dashed line). All data are mean ± standard error.

Ammonium was efficiently (>97%) removed (Appendix B.1) with effluent concentrations (i.e. concentration measured on the algal suspension withdrawn from the test tubes) ranging from < 1 mg NL^{-1} to 35 mg N L^{-1}. Maximum ammonium removal rate, assessed as the slope of the time trend of ammonium concentration under non-limiting ammonium concentration (> 10 mg N L^{-1}, data not shown) was found to be: 24±1.5 mg N L^{-1} d^{-1} for the three flasks. However, ammonium removal was also affected by other concomitant processes. Stripping is likely to have played a significant role since average pH was 9.6±0.6, leading to significant free ammonia levels. Moreover, nitrification was also observed although its appearance and relevance differed remarkably among each test tube and in time. As for nitrate, the average concentration slightly increased from day 24 onward from 2.1±0.2 to 5±0.4 mg N L^{-1} suggesting a slow nitrification process which was similar in all replicates (Appendix B.1). On the contrary, nitrite (Appendix B.2) remained negligible in replicate 1, while it increased remarkably in replicate 2 and 3 after day 60, reaching a maximum level of 210 and 110 mg N L^{-1}, respectively, at day 96; later, its concentration decreased. Denitrification was unlike to take place since dissolved oxygen was continuously entering the test tubes via aeration, maintaining aerobic conditions during both light and dark hours.

Extra phosphorus was dosed at time 33, 61 and 74 to increase the P concentration (Appendix B.1) in the test tubes from few mg P L^{-1} to 15-20 mg P L^{-1}. Average P removal rate was 0.5 mg P L^{-1} d^{-1}. Even for phosphorus, biological (assimilation/release) and chemical-physical (precipitation/dissolution) processes are expected to take place simultaneously.

Soluble COD in the liquid digestate increased during the first part of the test and eventually stabilized (Appendix B.1). The COD removal rate remained quite constant during the whole test, with average removal efficiencies of 83±4% which could be supported by heterotrophic bacteria or by mixotrophic algal growth (among others [29], [30], [31] and [32]).

As for the algal counts (Figure 5), *Chlorella* spp. was found to be the prevailing algal species (by up to 2 order of magnitude) in all vials up to day 68, then *Scenedesmus* spp. prevailed, probably due to a decrease in the ambient temperature under 20°C. The total number of algal cells was well correlated with the OD (R^2 of 0.77). The maximum total algal counts recorded were 1.3 E+07 cell mL^{-1} for replicate 1, 4.6 E+6 cell mL^{-1} for replicate 2, and 9.4 E+06 cell mL^{-1} for replicate 3; those values were obtained between day 52 and 68 when absorbance was at its maximum as well. The mean number of *Chlorella* spp. and *Scenedesmus* spp. reached in the test tubes was 2.5 E+6 cell mL^{-1} and 9.2 E+5 cell mL^{-1}. Replicate 1 had the larger algal growth, with a mean count of 4.1 E+6 cell mL^{-1} for *Chlorella* spp. and 1,1 E+0.6 cell mL^{-1} for *Scenedesmus* spp. Few rotifers, which feed on microalgae, were found in all the test tubes. Nematodes were found from day 33 in replicate 2, while in the other replicates they were found sporadically later on (from day 61 for replicate 1 and from day 90 for replicate 3).

3.3.2 Growth on adsorbed/stripped liquid digestate

The second set of algal culturing tests was conducted on the DIG_L after stripping (DIG_S) or stripping and adsorption (DIG_S&A), according to the procedure described in section 2.3.2. Three batches of DIG_S and DIG_S&A (B1, B2, B3) were prepared during the course of the experimentation and their characteristics are summarized in Table 2. Note that these three batches were sampled later than those used for digestate characterization, therefore their composition was slightly different from that reported in Table 1.

The adsorption step caused a remarkable reduction in both optical density (81±7%, similar to the removal efficiency obtained during the adsorption tests and reported in section 3.2). Similarly, high reductions were achieved for soluble COD (87±3%) and for nitrate (90±3%). On the contrary, no significant variation was observed in the ammonium concentration. As shown in Table 2, phosphorus was released by the activated carbon, leading to a remarkable increase in the phosphorus concentration in the DIG_S&A. This release depends on the AC activation process, which is based on the use of phosphoric acid. Indeed, activation with phosphoric acid is a well-established method for the preparation of activated carbons with optimal textural properties and porosity [33], [34] .

Of the three batches described in Table 2, B1 was used from the beginning of the culturing test until day 14, B2 from day 15 until 28, and B3 from day 29 until 40. The feeding procedure was similar to that applied to grow microalgae on the raw digestate. Since the ammonium concentration in the feed was lower, the average HRT, set according to the time needed for ammonium depletion, was much shorter than in the first set of trials, being 11 days up to day 29 and 8.8 days from day 30 to day 40.

Table 2: Characterization of the stripped (DIG_S) and stripped/adsorbed (DIG_S&A) digestate samples.

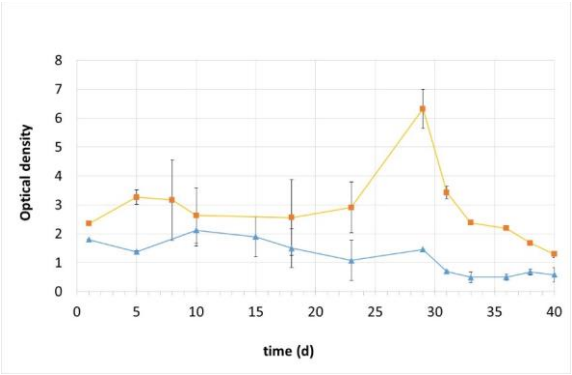
	DIG_S			DIG_S&A		
Batch #	B1	B2	B3	B1	B2	B3
Conductivity (mS cm^{-1})	3.5	4.7	4.2	3.6	4.2	4.7
OD (-)	0.27	1.04	0.98	0.04	0.28	0.14
$N\text{-}NH_4$ (mg L^{-1})	220	530	560	250	520	560
$N\text{-}NO_3$ (mg L^{-1})	9.3	10	10.5	1.0	0.68	1.3
Soluble COD (mg L^{-1})	1410	1370	1410	156	229	171
$P\text{-}PO_4$ (mg L^{-1})	3.4	5.65	6.3	588	280	367

According to the T test for paired data, daily biomass production (mg TSS L^{-1} d^{-1}) and biomass concentration (in term of optical density), was significantly different between algae grown on stripped digestate (DIG_S) and stripped and adsorbed digestate (DIG_S&A) (df = 10, p-value = 0.0001, and df=10, p-value= 0.0009, respectively for biomass production and biomass concentration). As shown in Figure 4a, microalgal concentration remained always higher in the test tubes fed on the DIG_S&A than in those fed on DIG_S, with differences becoming more and more significant with time. From day 15 to day 29, the optical density increased in the test tubes fed on DIG_S&A while it tended to decrease in those fed on DIG_S. This behavior suggests that a higher growth rate was achieved when feeding the stripped and adsorbed liquid digestate. Later, biomass concentration reduced in all the test tubes because of the reduction in the average HRT. The cumulated biomass production was computed from TSS data, and in Figure 4b it is compared to the cumulated volume of liquid digestate fed to the test tubes. The CBP appeared significantly higher in the test tubes fed on DIG S&A than in the other ones. Coherently, a significantly higher daily biomass production rate was observed in the test tubes fed on DIG_S&A (220±78 mg TSS L^{-1} d^{-1}) than on those fed on DIG_S (82±18 mg TSS L^{-1} d^{-1}). According to the T test for paired data, daily biomass production and biomass concentration obtained by feeding DIG_S and

DIG_S&A were significantly different (df = 10, p-value = p-value = 0.0001, and df=10, p-value= 0.0009, respectively for biomass production and biomass concentration).

A slightly higher removal efficiency of ammonium (Appendix C.1 and C.2) was observed, on average, in the test tubes fed on DIG_S&A (96±4%) than on those fed on DIG_S (88±9%).

a)



b)

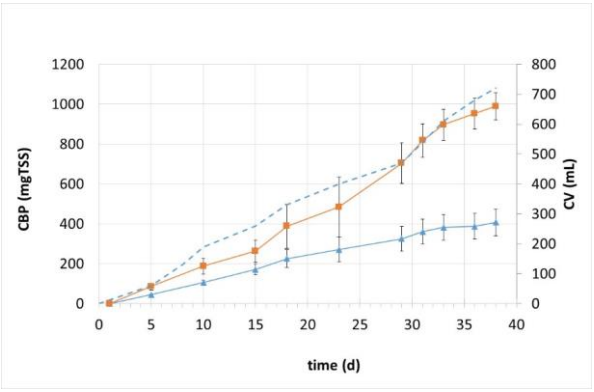


Figure 4. Results of the algal culturing tests on DIG_S (triangles) and DIG_S&A (squares): time trend of (a) optical density, (b) cumulated biomass production (CBP) and cumulated treated volume of pretreated digestate (CV, dashed line, on the secondary y-axis). All data are mean ± standard error.

Nitrification was almost negligible in the test tubes fed on DIG_S&A, while an increase in nitrite (up to 30 mg N L^{-1}) with no significant production on nitrate was observed in test tubes fed on DIG_S from day 30 onward (Appendix C.1 and C.2).

As for phosphorus (Appendix C.1 and C.2), very different concentrations were found in the DIG_S and DIG_S&A, as commented before. In the test tubes fed on DIG_S, effluent concentrations decreased from 3 to 1 mg P L^{-1} during the first two weeks; at day 15, extra P was dosed in order to increase the P level in each test tubes to 20 mg P L^{-1} thus avoiding P-limiting conditions. Later, P decreased slowly and, from day 20 onward, it stabilized around 5 mg P L^{-1}. In the test tubes fed on DIG_S&A, the P level remained between 200 and 300 mg P L^{-1}.

A limited COD removal (Appendix C.1 and C.2) was observed in the test tubes fed on DIG_S (15±3% on average), while a 30% increase in the soluble COD was observed in the test tubes fed on DIG_S&A. This indicates that the concentration of organic matter increased. This is probably due to the release of extracellular soluble organic material from the algal biomass that off-set its removal by algal or bacteria oxidation. Indeed, the residual organic matter after adsorption is expected to be poorly degradable.

As for algal counts (Figure 5), *Chlorella* spp. was found to be the prevailing algal species in the test tubes fed on DIG_S&A, while in the DIG_S ones the prevalent species was *Scenedesmus* spp. However, in all vials the number of both microalgae species was of the same order of magnitude. The total number of algal cells was well correlated with the optical density (R^2 between 0.8 and 0.9). The maximum values of total number of algal cells were obtained on day 10 and on day 29 in DIG_S and in DIG_S&A, respectively, when the maximum absorbance values were also observed. In those days, the total algal count was 2.8 E+06 cell mL^{-1} and 1.7 E+6 cell mL^{-1} for the two replicates of the DIG_S set, and 4.9 E+06 cell mL^{-1} and 5.3 E+06 cell mL^{-1} for the two replicates of the DIG_S&A set. The mean number of *Chlorella* spp. and of *Scenedesmu*s spp. in the DIG_S vials was 3.10 E+5 cell mL^{-1} and 4.33 E+5 cell mL^{-1}, respectively, while in the DIG_S&A vials it was 1.18 E+6 cell mL^{-1} and 9.59 E+5 cell mL^{-1}, respectively. Rotifers were found in both the sets, but in DIG_S their number was significantly larger than in the DIG_S&A one.

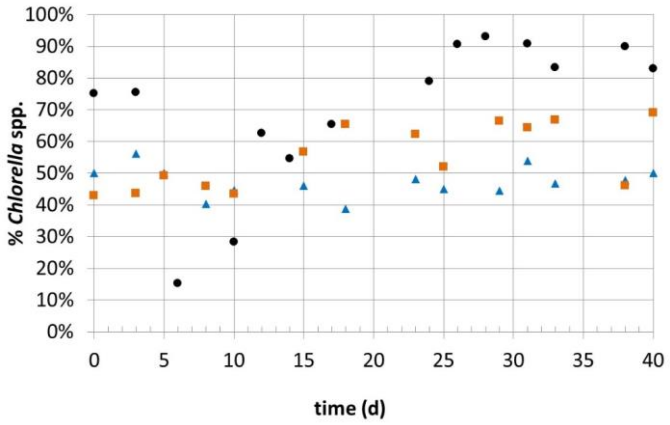


Figure 5. Average percent of *Chlorella* cells in DIG_L (rounds), DIG_S (triangles) and DIG_S&A (squares) culturing tests with time.

4. Discussion

Digestate is rich in nutrients that are needed in microalgal culturing. For this reason, the use of digestate for microalgal culturing has been proposed either as an opportunity to reduce microalgal-culturing costs or as a process to reduce the nutrient level in digestate, thus reducing the land request for digestate spreading, especially in areas of livestock intensive breeding. The dual-system "AD/microalgae" has recently attracted attention as an alternative use of the nutrient rich liquid fraction of digestate [6, 8, and 9] . Indeed, the liquid fraction of digestate contains high levels of ammonia nitrogen [3]. Nonetheless, some major limitations still exist to the industrial scale-up of microalgae ponds using the liquid fraction of digestate, which are mainly due to its high ammonium concentration and turbidity [2, 35]. Although ammonium is the preferable form for microalgal nitrogen uptake [36], high total ammonium nitrogen (TAN) (including both ammonium and free ammonia) levels may be inhibiting [3]. Turbidity is another important parameter that could limit the availability of photosynthetically active radiation, slowing down microalgae growth. Thus, dilution with fresh water is commonly applied to lower both the ammonium and turbidity levels, thus improving the efficiency of microalgae growth [13], but further affecting the overall economic and environmental balances of the dual

system. In the present study, a stripping step, which may allow for ammonium recovery, was applied to to the digestate to reduce the ammonium concentration, and further AC adsorption process was used for turbidity reduction in order to avoid the need for dilution.

Indeed, stripping is a well known chemical/physical process which allows for the recovery of nitrogen in the form of ammonium salt. Its applicability to the liquid fraction of digestate is still limited mainly due to the fact that the ammonium salts marketability is yet uncertain. The use of AC for turbidity reduction has been extensively investigated for wastewater or seawater treatment with good results [19] and [20]. The results reported in this study are in accordance with Hatt et al. [20], who noticed a minimum of 80% turbidity removal on secondary wastewater by performing batch tests at 20°C, for 2 h, under stirring conditions and by using different types of granular AC with a mean dosage of 0.4 g L^{-1}. Interestingly, Hatt et al. [20] highlighted a good correlation between the efficiency in turbidity reduction (assessed in terms of optical density at 400 nm) and the available meso and macropores of AC. As for the applied AC dose, the majority of studies that have investigated the turbidity reduction of effluents using activated carbon focused on wastewater with a much lower initial turbidity (around 7 NTU, [37]) which make AC dosages barely comparable to results of this experimentation. Indeed, the turbidity of digestate is much higher, with value around 1,063 NTU [38]. Therefore, higher AC dosages, ranging from 1 to 40 g L^{-1}, had to be applied in order to achieve relevant improvement in the optical density.

Nonetheless, the high production cost of AC remains a limitation for its full-scale industrial implementation and used in remediation process [39] and [40]. Thus, the use of AC produced from low cost lignocellulosic biomasses (i.e agricultural wastes) may be considered as a promising alternative in this specific application.

As shown in Table 2, the proposed pretreatments were successful in improving the treatability of digestate by microalgae. Indeed, the ammonium concentration is lowered by the stripping process down to acceptable levels (200-500 mg N L^{-1}). A digestate from the anaerobic digestion of waste sludge with similar ammonium level has been yet proven to be adequate for microalgal culturing [23]. However, stripping does not significantly affect the optical density, which is reduced by the adsorption process. Moreover, the applied pretreatments (stripping and adsorption) modify the N:P ratio, thus allowing for its potential optimization. Finally, as commented before, the optical properties are improved both in terms of optical density and of turbidity if compared to the original DIG_L.

Microalgal culturing tests were then performed to assess the impact of the applied pretreatments on the biomass growth rate. The raw digestate could be successfully used to grow microalgae and a microalgal production rate of 27±13 mg TSS L^{-1} d^{-1} was obtained. In the literature, a large interval of microalgal growth rates can be found, spanning from 30 to 670 mg TSS L^{-1} d^{-1}, and this is partly due to differences in the culturing mode, light intensity, algal strains, and digestate pretreatments [2] and [3]. The low value obtained in this experimentation can be partially explained by the high free ammonia concentrations and by the high digestate optical density, which limits light availability. In order to limit ammonia inhibition, a quite high hydraulic retention time had to be applied (around 100 days, on average, with lower values of 55 d during the stationary growth phase). Such a long HRT would result in huge pond volumes and the land request for the algal pond could become prohibiting.

By applying pretreatments, the biomass production rate was indeed improved since it increased to 82±18 mg TSS L^{-1} d^{-1} when the stripped digestate was used and to 220±78 mg TSS L^{-1} d^{-1} when a further adsorption step on activated carbon was applied, which corresponds to an almost 10 fold increase if compared to results obtained on the raw DIG_L. A similar increase in the areal productivity can therefore be expected leading to a proportional reduction in land request for microalgal culturing.

Further studies with an AC activated without the use of phosphoric acid would be needed to confirm that the positive results obtained by the AC pre-treatment depend chiefly on the reduction of the OD of the sample, and thus on the better light penetration, or if phosphorus release played a significant role. Finally, the feasibility of applying the sole adsorption on raw digestate, without ammonia stripping deserves to be further investigated to clarify whether the combination of adsorption and stripping lead to an additive response on the algal productivity which is a relevant information in view of process scale-up.

The use of pretreatments appears to affect the composition of the community of microalgae and bacteria that develops. Simultaneous activity of microalgae and nitrifying bacteria took place in microalgae cultivation tests with both raw DIG_L and DIG_S, while nitrification was not observed by feeding DIG_S&A. When nitrification occurred, nitrite accumulation up to 210 mg N L^{-1} were registered indicating the presence of ammonia oxidizing bacteria activity, but this phenomenon was not stable and the trigging factors are not yet clear. Nitrate accumulation was not observed, indicating a negligible activity of nitrite oxidizing bacteria (NOB).

Significant nitrite accumulation was observed when free ammonia levels approached and overcame 50 mg N-NH_3 L^{-1}. These high free ammonia levels probably led to an inhibition of NOB, which are known to be sensitive to free ammonia already at concentration of 1-5 mg N-NH_3 L^{-1}, with an IC50 (50% inhibition concentration) of 10 mg N-NH_3 L^{-1} [41]. On the contrary, ammonia oxidizing bacteria (AOB) are more tolerant to high free ammonia levels, as suggested by the higher IC50 values reported in the literate and varying within the range of 40-600 mg N-NH_3 L^{-1} [42], depending on acclimation. Nitrification is indeed a common process taking place in algal ponds/photobioreactors treating digestate. In an open pond treating digested piggery slurry, Gonzalez-Fernandez et al. [43] verified that nitrification (with mainly nitrite accumulation) was the major nitrogen transformation process. Marcilhac et al [28] reported that microalgae could outcompete nitrifying bacteria since the nitrification rate decreased with the increase in microalgal N assimilation. Moreover, their experiments showed how microalgae became stronger competitors for nutrients under P-limiting (<0.1 mg P L^{-1}) conditions. Uggetti et al. [9] reported the presence of nitrification in their microalgal growth tests and observed that nitrite and nitrate production was enhanced at high microalgae concentration (1.3 or 1.8 g TSS L^{-1}). This evidence was explained by the fact that, at higher algal concentration, larger quantity of oxygen are produced, stimulating ammonium oxidation by nitrifiers, while microalgal growth rate is negatively affected by a self-shading phenomenon. In the present experimentation, the only condition in which negligible nitrification activity was observed was the use of DIG_S&A as feed. This same conditions also led to the highest microalgal production rate and biomass concentration. This observation is in disagreement with data from Uggetti et al. [9]. This may be due to the fact that in our experiment air was supplied continuously, leading to constantly non-limiting dissolved oxygen levels. Moreover, in test tubes fed on the adsorbed digestate, the lower OD of the DIG_S&A allowed the culture to achieve a higher algal concentration before it possibly became light-limited. Despite being a commonly observed process, the true mechanisms and the driving forces of the competition between microalgae and nitrifiers still need to be fully elucidated. Nonetheless, the use of adsorbed digestate suppressed nitrifiers growth suggesting that the lower OD and higher P level in DIG_S&A contributed to favor microalgae over nitrifiers.

As previously mentioned, the removal of the soluble COD was also very different from one case to another, reaching 83% in DIG_L, just 15% in DIG_S and being negative, i.e. with a 30% increase, in DIG_S&A. Of

course, the starting COD was much higher in DIG_L, lower in DIG_S and minimum in DIG_S&A; moreover, pre-treatments are likely to have removed the most easily degradable fraction of the organic matter in the digestate. Actually, the residual concentration of organic matter in algae/bacteria consortia is the result of the combined effect of bacterial oxidation, algal degradation and release from either production of exopolymeric substances or death cells lyses. Moreover, many authors reported effective mixotrophic growth of algae, such as various species of *Chlorella*, accounting for strong COD removal also in wastewaters containing poorly degradable organic matter, pointing out the double role of organic compounds as both carbon source and growth factors (among others [29, 30, 31, and 32]). On the contrary, mixotrophic metabolism is rarely reported for *Scenedesmus* spp. [44]. In the three sets of algal culturing tests, some differences exist in the percentage of *Chlorella* cells, as shown in Figure 5. Specifically, the relative abundance of *Chlorella* spp. decreases from DIG_L to DIG_S and then to DIG_S&A, as the efficiency of COD removal in the three cases, suggesting that the abundance of *Chlorella* spp. could be depend on the availability of organic matter and could have contributed to the higher COD removal.

5. Conclusions and perspectives

Results of this experimentation suggest the feasibility of culturing microalgae on the liquid fraction of digestate without the need for dilution. However, long hydraulic retention times had to be used when using the raw DIG_L and low biomass productivity was obtained. In view of reducing the land request, pretreatments (stripping/adsorption) were tested as a mean to sustain faster microalgal grow. Commercial activated carbon proved to be effective in optical density reduction. By feeding the pretreated digestate, much faster microalgal growth was obtained. Indeed, these pretreatments allowed to increase the microalgal production rate by almost 10 times. Nonetheless, the high production cost of AC remains a limitation for its full-scale industrial use. Therefore, AC produced from renewable biomasses like lignocellulosic substrates may be considered as a perspective alternative in this specific application.

Author contribution

All authors have contributed to the conception, design and data acquisition of the experimental phase and to data analysis, drafting and revising of the manuscript.

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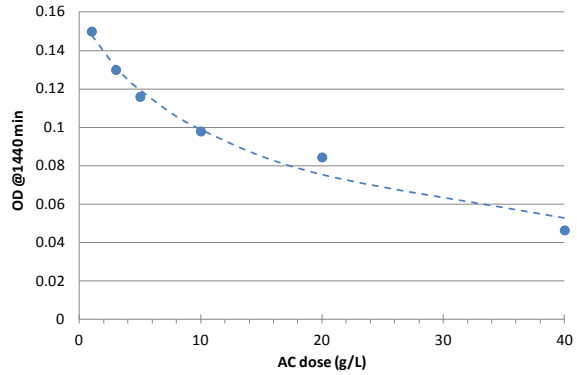
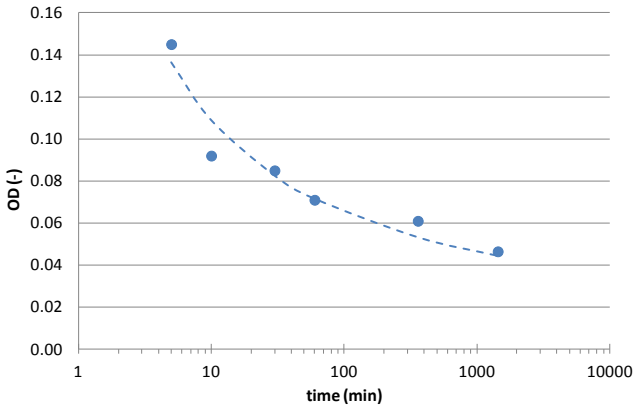
Appendix A.1: Effect of the AC dose on the optical density measured at equilibrium in long term adsorption tests (24 h). Data are fitted with an hyperbolic curve (dashed line: $y = 1.817/(11.28+x^{0.8518})$ with $R^2 = 0.979$).



Appendix A.2: OD time trend during a long term adsorption test with a AC dose of 40 g L^{-1}. Data are fitted with an hyperbolic curve (dashed line: $y = 6.14\ 10^{-4}/(x^{0.00166}-0.998)$ with $R^2 = 0.929$).



Appendix B.1: Effluent concentrations in microalgal growth tests on raw digestate (DIG_L). Mean and standard deviation refer to triplicate data.

Time	COD		Nitrate		Nitrite		Ammonium		Phosphate	
(d)	(mg L^{-1})		(mgN L^{-1})		(mgN L^{-1})		(mgN L^{-1})		(mgP L^{-1})	
	mean	std	mean	std	mean	std	mean	std	mean	std
10	2	0.2	2.6	1.21	0.38	0.35	0.13	0.11	2.4	3.6
24	4	0.1	0.6	1.08	0.09	0.16	6.7	5.3	0.11	0.01
31	7	0.2	2.2	0.20	0.08	0.01	17	28	0.28	0.05
38	11	0.4	2.7	0.10	0.26	0.30	12	21	9.0	4.3
45	16	0.5	2.3	0.09	0.08	0.01	0.32	0.42	4.2	2.6
52	19	1.1	3.1	0.29	0.93	1.40	0.33	0.49	2.1	1.2
54	24	1.4	3.4	0.10	2.4	2.8	38	5	0.32	0.11
61	28	1.9	3.5	0.15	0.24	0.41	0.06	0.10	1.4	1.5
66	34	2.2	4.1	0.29	4.90	4.23	16	20	6.4	7.5
70	40	2.5	4.7	0.28	18	22	22	19	2.6	2.3
74	51	2.7	4.5	0.25	47	49	12	14	6.4	6.6
90	58	2.7	5.0	0.40	60	56	31	53	22	1.4
102	65	2.5	4.4	0.39	57	57	5.6	10	10	6.3

Appendix B.2: Effluent nitrate concentrations from each replicate test vial (T1, T2, T3), in microalgal growth tests on raw digestate (DIG_L).

Time	**Nitrite (mgN L^{-1})**		
(d)	T1	T2	T3
10	0.03	0.72	0.38
24	0.00	0.00	0.27
31	0.09	0.07	0.08
38	0.10	0.61	0.07
45	0.08	0.08	0.06
52	0.25	0.00	2.5
54	0.01	5.4	1.7
61	0.00	0.00	0.72
66	0.02	7.3	7.4
70	0.20	10	43
74	0.09	43	98
90	0.07	67	112
102	0.08	56	113

Appendix C.1: Effluent concentrations from each duplicate test vial (T1, T2), in microalgal growth tests on stripped digestate (DIG_S).

Time	COD		Nitrate		Nitrite		Ammonium		Phosphate	
(d)	(mg L^{-1})		(mgN L^{-1})		(mgN L^{-1})		(mgN L^{-1})		(mgP L^{-1})	
	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
5	1100	1201	8.5	15.72	1.7	37	4.1	26	2.6	2.4
10	1136	1065	9.31	8.25	0.05	10	0.54	10	1.0	1.0
15	1232	1151	10.2	10.2	0.42	0.48	0.31	29	0.9	1.0
18	1380	1400	10.7	10.4	8.6	0.18	17	45	15	11
23	1136	1217	9.56	10.3	8.2	3.0	8.3	59	9.4	16
29	1140	1183	9.78	9.08	6.2	4.1	96	33	3.4	4.9
31	1240	1080	9.81	14.13	19	0.9	63	143	5.4	5.3
33	1260	1230	6.52	5.54	23	11	124	210	4.6	4.1
36	1110	1310	12.64	11.86	30	27	40	255	18	4.7
38	1170	1090	15.14	9.92	24	32	42	82	9.7	5.1
40	1100	1070	10.54	9.08	21	34	27	68	3.7	5.0

Appendix C.2: Effluent concentrations from each duplicate test vial (T1, T2), in microalgal growth tests on stripped and adsorbed digestate (DIG_S&A).

Time	COD		Nitrate		Nitrite		Ammoniacal N		Phosphate	
(d)	(mg L^{-1})		(mgN L^{-1})		(mgN L^{-1})		(mgN L^{-1})		(mgP L^{-1})	
	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
5	n.a.	n.a.	0.60	0.54	0.04	0.00	n.a.	18	334	379
8	14	15	0.7	0.8	0.0	0.0	n.a.	71	287	310
10	26	28	0.8	0.9	0.0	0.0	n.a.	20	267	258
15	44	49	1.0	1.1	0.0	0.0	2	7	218	241
18	66	73	0.9	1.1	0.0	0.0	0.2	0.1	290	315
23	84	90	0.8	0.8	0.1	0.0	0.0	0.0	299	390
29	101	109	0.9	0.8	0.0	0.0	16	6	207	209
31	119	127	0.8	0.7	0.1	0.0	24	39	216	228
33	135	142	0.8	0.6	0.5	0.1	0.3	17	268	277
36	143	157	1.0	0.6	0.4	0.0	14	77	208	262
38	153	164	1.5	0.6	1.1	0.1	8.3	37	241	193
40	161	172	0.7	0.5	0.4	0.0	0.0	0.0	227	240