**Biochemical methane potential of oil-extracted microalgae and
glycerol in co-digestion with chicken litter**

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Abstract

The objective of this work was to evaluate the technical feasibility of using both oil-extracted microalgae (M) and glycerol (G) in co-digestion with chicken litter (CL), thereby improving biochemical methane potential (BMP). Different feedstock ratios of M (0 - 30 %), G (0 - 3 %) and CL (67 – 100 %) were investigated to determine the best co-digestion condition under mesophilic conditions. According to the modified Gompertz model, the best BMP (131.1 mL CH₄ g VS_{fed}⁻¹) was obtained with the triple co-digestion (M:G:CL) in a proportion of 30:3:67. This yielded a methane production rate (μ_m) of 3.3 mL CH₄ g VS_{fed}⁻¹ d⁻¹ and a lag time (λ) of 17.4 d. This treatment reduced chemical oxygen demand (COD) by 91.02 % and increased the methane yield 15.8 % with respect to the CL control.

Keywords: oil-extracted microalgae; glycerol; VFAs profile; Gompertz model; biodiesel residuals; mesophilic.

1 Introduction

The microalga *Chlorella vulgaris* has received significant attention recently due to its fast growth rate and consequently its high biomass productivity (15 to 30 g m⁻² d⁻¹) (Yang et al., 2015; Yuan et al., 2012). It is a rich source of protein (51 -58 % dry basis) and lipids (14 – 22 % dry basis) (Sialve et al., 2009). Since biodiesel production from microalgae has two main residuals, glycerol and oil free microalgae biomass, it has been proposed that these residuals can be used as a feedstock for methane production through anaerobic digestion. Using current methods for biofuel production from microalgae, it is estimated that 60-70 % of the starting biomass could be recovered (Ward et al. 2014). In addition, the use of these feedstocks can improve methane production, in particular, when they contribute to balancing the C:N ratio of nutrient-poor substrates. Therefore, Ward et al. (2014) proposed that anaerobic digestion with microalgae could be a key unit process that integrates efficiency into the production of microalgae-derived biofuels.

Glycerol has been evaluated as a substrate for anaerobic digestion and found to either improve or reduce methane yield depending on glycerol quality and co-substrate mixes. For example, Fountoulakis and Manios (2009), working with the organic fraction of municipal solid

waste and a mixture of olive mill wastewater and slaughterhouse wastewater, reported that the addition of 1% glycerol enhanced methane production. However, another research group (Siles López et al., 2009), comparing acidified and distilled glycerol in co-digestion with granular and non-granular sludge, found that methane yield fell when the amount of acidified glycerol was increased, but when distilled glycerol was used, methane yield either increased or decreased depending on glycerol percentage. When glycerol was co-digested with maize silage, maize grain and pig manure, it was reported that the maximum methane production was with 6 % glycerol while higher glycerol concentrations up to 15 % gave lower yields (Amon et al., 2006b).

Methane production has also been evaluated using different microalgae residuals in co-digestion with different substrates. Some examples of methane yield, expressed in $\text{mL CH}_4 \text{ g}^{-1} \text{VS}_{\text{fed}}$, are presented as follows: poultry manure and the microalga *Scenedesmus*, 112.7 (Nasr et al., 1983); maize silage, maize kernels, pig manure and glycerol, 365 to 439 (Amon et al., 2006a); *Scenedesmus* sp., *Chlorella* sp. and paper, 292 (Yen & Brune, 2007); *Chlorella* sp. biomass residues and glycerol, 302 (Ehimen et al., 2011); and poultry manure and glycerol, 480 (Chow et al., 2015). Zhen

et al. (2016) explored the co-digestion of mixed microalgae (MM) (*Scenedesmus sp.* and *Chlorella sp.*) and food waste (FW) in the range 0 to 100 % microalgae. They found that the best performance was with a MM:FW ratio of 20:80. Ehimen et al. (2009) evaluated methane production using microalgae biomass residuals from biodiesel production as well as co-digestion with glycerol. These authors reported that methane increased from 4 to 7% for co-digestion of glycerol with microalgae residues. In additional work, Ehimen et al. (2011) explored the influence of hydraulic retention time (HRT), C:N ratio, and total solids (TS) on methane production using co-digestion of microalgae residues with glycerol under mesophilic conditions. Their main findings indicated that in the C:N ratio range of 5 to 25, maximum methane production was obtained with a 12.44 value which was 50 % greater yield than microalgae alone. Alzate et al. (2014) reported that methane production of oil-extracted microalgae was higher than the non-extracted type. They attributed this performance due to the fact that lipid-extraction constituted a pretreatment that increased the biochemical methane potential of microalgae.

Taking into consideration the previous results, and since there are no reported data on co-digestion of mixed biodiesel production residuals, the contribution of the present work was to evaluate the

technical feasibility of using both glycerol and oil-extracted microalgae in co-digestion with chicken litter to improve biochemical methane potential (BMP). The influence of different feedstock ratios was investigated to determine the best co-digestion conditions. The experimental data were fitted to the modified Gompertz model (Kim et al., 2003; Olsson et al., 2014; Zwietering et al., 1990) to obtain the BMP.

2 Materials and Methods

2.1 Experimental procedure

Three different feedstocks were used as follows: glycerol grade United States Pharmacopeial Convention (USP); oil-extracted microalgae biomass from *Chlorellavulgaris* powder (FutureFoods Company, Mexico); and chicken litter (from a commercial poultry farm in Tepetlaoxtoc, Mexico). The characteristics of the feedstocks are summarized in **Table 1**.

...**Table 1**. Physical and chemical characteristics of substrates.

The experiment was performed in serum bottles as microcosms with a capacity of 305 mL and a working volume of 250 mL each. Inoculum

was added at a ratio of 10% (v/v). The inoculum was obtained from a mesophilic laboratory digester, which was operating for one year and fed with chicken litter as sole substrate at 3 % TS. The treatments, including different ratios of microalgae (0 - 30 %), glycerol (0 - 3 %) and chicken litter (67 – 100 %) in aqueous solution, were prepared in triplicate. The treatment characteristics are presented in **Table 2**. The slurry was obtained by adding water to feedstock and filtering the resulting solution in order to remove large particles. All of the treatments had 3 % TS. Microcosms were sealed with a rubber septum and metal cap, and were purged using nitrogen gas to displace oxygen. Incubation was done at 37.7 ± 1 ° C. Assays were conducted for 90 days.

Table 2. C:N ratio, pH, TS, VS and chemical composition of the feed for each treatment.

2.2 Analytical methods

For the substrates, TS, volatile solids (VS), fixed solids (FS), and COD were determined using the standard methods of APHA (1998), while ammonia nitrogen was determined with Method 10031 (HACH, 2004). pH was measured with a potentiometer (Thermo Scientific Orion 5 Star, Singapore). Profiles of volatile fatty acids (VFAs) were obtained for acetate, propionate, butyrate, iso-butyrate, valerate, iso-valerate,

caproate, iso-caproate, and heptanoate acids using gas chromatography (GC) (Claurus 500, Perkin Elmer, USA) equipped with a flame ionization detector (FID) and a capillary column Elite-FFAP with a length of 30 m and 0.32 mm diameter (Perkin Elmer, USA). VFAs were determined using a sample of effluent which was acidified to pH 3 with HCl and centrifuged at 14,500 rpm for 10 min (MiniSpin Plus model micro-centrifuge Eppendorf, Germany). The supernatant was injected with an auto-sampler using 5 μ L volume. Operation conditions were as follows: gas flow 5 mL min⁻¹ at 10.6 psi, 150 °C injection port, 100 °C oven for 8 min with ramp of 160 °C for 8.5 min, 250 °C detector, and retention time of 16.5 min. Helium was used as the carrier gas. Free Volatile Acid Mix analytical grade (46975-U, Brand Sigma-Aldrich, USA) was used as a standard. Elemental analysis (C, H, N, S) was performed with a Perkin Elmer PE2400 analyzer; the standard compound was cysteine (analytical reagent, Perkin Elmer).

Protein content was determined with the micro-Kjeldahl procedure according to standard methods (APHA, 1998). The ether extract content was obtained with hexane in a Soxtec System (Foss model 2050, Spain). Ash was determined with FS. Carbohydrates were estimated as the remaining fraction of TS following removal of protein, lipids, and FS. Crude fiber was determined with method ISO 5498. Water

determination in glycerol was done using Karl Fischer colorimetric titration method USP 28-NF 23.

Biogas production was quantified by the displacement method in saline water 10 % (v / v). Methane content was evaluated using a GC (Claurus 500, Perkin Elmer, USA). 10 µL biogas samples were injected into the GC with the following conditions: flow 14 mL min⁻¹ at 14 psi, 100 °C injection port, 70 °C oven, and 100 °C detector. The retention time was 4 min; helium was used as the carrier gas. The percentage of methane was obtained using a calibration curve with pure methane (HDSP No. P-4618-F, Praxair, Mexico). Methane percentage is reported with normal conditions of temperature and pressure.

2.3 Modeling and statistical analysis

The methane production results were fitted to the modified Gompertz model (Kim et al., 2003; Olsson et al., 2014; Ortega-Martinez et al., 2016; Zwietering et al., 1990) which is presented in **Equation 1**.

$$AMY = BMP \cdot \exp \left\{ - \exp \left[\frac{\mu_m \cdot e}{BMP} (\lambda - t) + 1 \right] \right\} \quad (1)$$

AMY is the accumulated methane yield (mL CH₄ g VS_{fed}⁻¹) at time t (d), BMP is the biochemical methane potential (mL CH₄ g VS_{fed}⁻¹), μ_m is the

rate of methane production per day ($\text{mL NCH}_4 \text{ g VS}_{\text{fed}}^{-1} \text{ d}^{-1}$), e is the mathematical constant (2.718282), λ is the time lag phase (d), t is the time of digestion (d). All regression models were fitted using SigmaPlot 12.5 version. A weighted principal component analysis (PCA) was used to evaluate the results of the feed mix C:N ratio, pH, TS, VS, COD, N-NH₃ and the modified Gompertz model BMP , μ_m , and λ . PCA was also used to evaluate the profiles of the three main fatty acids (acetate, propionate, and butyrate) along the entire digestion period. PCA was performed with SAS package version 9.1 (SAS Institute Inc., Cary, NC, USA).

3 Results and Discussion

3.1 Biochemical methane potential

Figure 1 shows the accumulated methane produced in all of the treatments for 90 days. In all cases, three typical periods can be defined: lag phase, exponential methane production and asymptotic methane accumulation. Methanogenesis in this microbiome is expected to occur through the aceticlastic pathway because this is a mesophilic system (Batstone et al., 2002). The formation of acetate in mesophilic digestion primarily occurs through fermentation of carbohydrates (acidogenesis). Additional acetate could also arise, though, from fermentation of amino acids and the oxidation of long chain fatty acids

(Batstone et al., 2002). The initial delay in methane accumulation for about 10 days in all of the microcosms could be attributed to oxygen contamination which will inhibit methanogenesis until oxygen is removed by facultative anaerobes(Botheju & Bakke, 2011). During the second period, methane was produced due to the consumption of the large, rapidly rising, acetate pool. In the third period, following the initial decline of acetate levels, methane production appears to be based on the breakdown of a rising propionate pool which was converted into additional acetate. When methane production decreased during the final 20-30 days, acetate accumulation again increased reflecting the decline in aceticlastic methanogenesis.

... **Figure 1**.Accumulated methane yield and VFAs profile during assays.

PCA applied to acetate (A), propionate(P), and butyrate(B) is presented in **Figure 2**, where A_i , P_i , and B_i are the fatty acid concentrations at i days after the assays started. The variations in the results are explained as 37.9 % by Component 1 and 20.5 % by Component 2. The variation in Component 1 is positively related to B70, P70, and B50 and negatively to B90 and P40, while Component 2 is mostly influenced by P10,B10, and A10. In **Figure 2**, it is clear that PCA can decrease data

dimensions and make data interpretation easier; thus it is possible to see that the treatment with CL is separated from the other treatments. In the case of the double co-digestions (G-CL or M-CL), PCA placed them to the right of the control while the triple co-digestion treatments (M-G-CL) were displayed in the top left of the plot. This analysis shows that the VFA profiles of these treatments do have broad similarities, which is probably because it is based on metabolites (VFAs) representing the central position in the methanogenic food web (acidogenesis and acetogenesis) which has less microbial and metabolic diversity than the initial hydrolytic stage (Campanaro et al., 2016). However, some differences in VFA profiles were also evident. In order to clarify the trends, VFAs were plotted individually in **Figure 3**. Thus, it was possible to recognize four different behaviors as follows:

- **CL mono-digestion.** This treatment has a unique behavior compared to the other treatments (Figure 3 a,c,e). Acetate concentration presents a unimodal distribution with the highest peak on day 17, corresponding to the end of the lag time, followed by a steep decline between days 15 and 30, and then slows leading to complete depletion. The maximum propionate concentration occurred at day 30 followed by a complete reduction by day 60.

The consumption of butyrate began at day 10 and ended by day 30.

- **G-CL co-digestion.** The acetate profiles of both treatments were similar: both had a maximum concentration on day 17 which was a few days before the end of the lag phase, while one showed a bimodal distribution (Figure 3 a,c,e). Propionate exhibited a bimodal distribution with two similar maximum concentrations on days 17 and 50. The complete reduction of propionate occurred on days 80 and 90 for M:G:CL ratios 0:3:97 and 0:1.5:98.5, respectively. For butyrate, the maximum of both treatments was on day 17.

- **M-CL co-digestion.** The patterns of VFAs are quite similar to the G-CL co-digestion (Figure 3 a,c,e). The acetate profiles of both treatments are alike showing a bimodal distribution and a maximum concentration on day 17. Propionate presented a bimodal distribution with two similar maximum concentrations on days 17 and 50, and complete consumption by day 70. The maximum butyrate concentration of both treatments was on days 17 for 30:0:70 and 20 for 15:0:85.

- **M-G-CL co-digestion.** The six treatments had similar behaviors (Figure 3 b,d,f). Acetate concentration presented a

unimodal distribution in all treatments except the M:G:CL ratio of 10:1:89 which had a bimodal distribution. The first high peak for the 5 similar treatments was on day 10 and the second one was on day 80; for the other treatment, the high peaks were on days 22 and 65. The propionate concentration also showed bimodal distribution in all the treatments. The first high peak occurred from day 10 to 17 while the second peak occurred from day 30 to 40. Butyrate profiles were not well defined: three treatments showed a unimodal distribution, while three others showed a bimodal distribution. Acetate was completely processed by day 90. Propionate uptake was completed by day 40 for 5 treatments and by day 50 for one. Butyrate had the lowest concentration after day 40 for five treatments and after 50 days for the other.

Figure 2. Principal component analysis based on VFAs profiles.

Figure 3. Acetate, propionate and butyrate patterns along BMP evaluation.

The above patterns have several common characteristics. Within the first 10-20 days, acetate, propionate and butyrate levels increased quickly. The majority of the initial acetate pool was probably created by the fermentation of carbohydrates, and further supplemented by

the oxidation of butyrate and propionate. Carbohydrates were present in both the chicken litter and microalgae. The readily degradable carbohydrates (hexoses and pentoses) in the microalgae biomass could be fermented into these three fatty acids. The conversion of glycerol to acetate and butyrate is also likely to have occurred during this time (Viana et al., 2012). In addition, each of the treatments showed two peaks of propionate. We propose that the first peak corresponded to the initial carbohydrate fermentation while the second peak may have been due to fermentation of lactic acid (Ren et al., 1997). In a mesophilic digester treating glycerol, Ferguson et al. (2016) found that lactic acid was the major fermentation product within three days of glycerol co-digestion. However, the presence of lactic acid was not measured in our study and requires additional experimental confirmation. In contrast to all of the treatments, the control microcosms (100% CL) showed a decline in fatty acids during the first 10 days which could be attributed to reduced levels of easily degraded carbohydrates.

Table 3 shows the average of the modified Gompertz model parameters applied to the individual replicates. In the table are also shown the coefficients of determination for each replicate. The BMP and μ_m showed

a slight trend to fall as the C:N ratio rises while the lag phase showed an opposite tendency to increase. In the case of BMP, almost all of the treatments showed a better BMP than the control. Comparing the treatments showed that the control (T11) produced 113.2 mL CH₄ g VS_{fed}⁻¹, while M-CL co-digestions T2 and T6 produced 129.6 and 117.8, respectively. This indicated a trend where higher ratios of microalgae enhanced BMP. In the case of co-digestion with glycerol, BMP showed a different pattern compared to the control: the lowest amount of glycerol (T10) was associated with reduced BMP (97.4 mL CH₄ g VS_{fed}⁻¹), while the highest amount of glycerol (T9) was associated with higher BMP (116.7). Similar results have been reported by Siles Lopez et al. (2009).

Some research groups have found that methane yield from microalgae can be enhanced by using pre-treatments or co-digestion. For example, microalgae as sole substrate was found to produce between 82.1 (Passos et al., 2016) and 88.0 mL CH₄ g VS⁻¹ (Zhen et al., 2016). In the former, the yield was increased by 75.2 % when a pre-treatment with 0.5 % KOH at 80 °C for 2 h was applied, while in the latter, the yield was increased when co-digestion with microalgae: food waste (20:80) was used.

In general, and based on the data in **Table 3**, we found that every triple co-digestion with M-G-CL and double co-digestion with M-CL enhanced methane production compared to the control. In contrast, co-digestion with G-CL showed either a decrease (14.0 %) or an increase (3.0 %) of BMP depending on G:CL ratio. The BMPs of M-G-CL were in all cases superior to the control (2.0 to 15.8 % higher). The highest yield (131.1 mL CH₄ g VS_{fed}⁻¹) was obtained with T1 (30:3:67) which can be attributed to the fact that the treatments with oil-extracted microalgae have a higher concentration of carbohydrates than chicken litter (**Table 1**). Thus, the substrates in the triple co-digestion are more accessible to microbial activity and consequently available for the acid-genesis and aceto-genesis. These results are in concordance with the composition of the microcosms after 90 days (**Table 4**). The greatest reduction of TS was made by the treatments that had the best yield, T1 (30:3:67) and T2 (30:0:70) with 38.8 and 37.3 % BMP increases, respectively. In the case of COD, all of the treatments reached about 90 % reduction. The ammonia levels rose from 17.8 to 94.5 %, having a maximum concentration of 776 mg L⁻¹, which is below the concentration for methane production inhibition.

...**Table 3**. Modified Gompertz model parameters determined from the experimental data.

...**Table 4**. Chemical composition of digestate after 90 days.

PCA applied to the initial composition (C:N, pH, TS, VS, COD, N-NH₃) and the modified Gompertz model (BMP, μ_m , and λ) is presented in **Figure 4**. The variations in the results are explained as 51.4 % by Component 1 and 24.8 % by Component 2. The variation in Component 1 is positively related to VS and BMP and negatively to C:N, while Component 2 is mostly influenced by TS and N-NH₃. In **Figure 4**, the treatments using G-CL were completely separated from the other treatments which showed lower VS, BMP, TS and N-NH₃. In addition, the treatments with M-CL were located along the Y axis of the plot and mainly showed changes in VS and BMP. These two groups are completely separated from the control (CL) which showed high levels of TS and N-NH₃. In the case of the triple co-digestion (M-G-CL), the values are spread along the plot. However, there is a trend for groups to form with the treatments that have similar TS and BMP which is similar to what was found by the modified Gompertz model.

...**Figure 4.**Principal component analysis based on initial chemical composition and BMP.

4 Conclusion

In most cases, BMP from co-digestion with oil-extracted microalgae, glycerol and chicken litter under mesophilic conditions had a better yield than mono-digestion with chicken litter. M-G-CL increased BMP (2.0 to 15.8 %), M-CL also increased BMP (4.1 to 14.1 %); G-CL showed either an increase (3.0 %) or decrease (14.0 %) depending on G:CL ratio. The 30:3:67 M:G:CL treatment (6.94 C:N) had the highest yield (131.1 mL CH₄ g VS_{fed}⁻¹). The VFA profiles showed differences among the three co-digestion types and the control, suggesting differences in the availability of easily degraded substrate molecules and pathway kinetics depending on feed composition.

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FIGURE CAPTIONS

Figure 1. Accumulated methane yield and VFAs profile during assays.

Figure 2. Principal component analysis based on VFAs profiles.

Figure 3. Acetate, propionate and butyrate patterns along BMP evaluation.

Figure 4. Principal component analysis based on chemical composition initial and BMP.

- Microalgae *Chlorella vulgaris*
- Glycerol
- Chicken litter

Microcosms
37 °C, 90 d

Biogas, CH₄, COD,
TS, VS, NH₃, pH, VFA's

$$AMY = BMP \cdot \exp \left\{ -\exp \left[\frac{\mu_m \cdot e}{BMP} (\lambda - t) + 1 \right] \right\}$$

BMP

λ

μ_m

Figure 1

Figure 2

Figure 3

Figure 4

Table 1. Physical and chemical characteristics of substrates.

Parameters	Unit	Substrates		
		Microalgae	Glycerol	Chicken litter
TS	[%]	93.65 ± 0.19	99.86	66.28 ± 1.16
VS/TS	[%]	92.75 ± 0.03	NA	69.61 ± 0.71
Protein	[% D.B.]	60.13 ± 0.03	NA	24.70 ± 2.13
Extract ether	[% D.B.]	0.97 ± 0.18	NA	1.81 ± 0.15
Ash	[% D.B.]	6.79 ± 0.02	NA	20.13 ± 0.12
Carbohydrates	[% D.B.]	25.76 ± 0.25	NA	19.65 ± 3.52
Crude fiber	[% D.B.]	3.81 ± 0.24	NA	16.25 ± 0.37
C	[% w/w]	50.27 ± 0.05	38.79 ± 0.15	34.86 ± 1.28
H	[% w/w]	7.02 ± 0.11	7.97 ± 0.63	4.80 ± 0.19
N	[% w/w]	9.40 ± 0.03	0.72 ± 0.05	4.28 ± 0.08
S	[% w/w]	1.61 ± 0.20	0.57 ± 0.12	0.44 ± 0.04
C:N	[-]	5.35 ± 0.02	53.62 ± 3.34	8.15 ± 0.21

NA: not analyzed.

Table 2. C:N ratio, pH TS, VS and chemical composition of the treatments' feed.

No.	Substrates			C:N	pH	TS	VS/TS	COD	N-NH ₃
	M	G	CL						
						[% D.B.]	[%]	[mg L ⁻¹]	[mg L ⁻¹]
1	30	3.0	67.0	6.94	7.99	2.98 ± 0.04	78.2 ± 0.1	18416 ± 3858	475 ± 38
2	30	0.0	70.0	6.79	7.96	3.05 ± 0.04	78.6 ± 0.1	18416 ± 4409	416 ± 65
3	25	2.5	72.5	7.10	8.15	2.97 ± 0.01	77.5 ± 0.2	13416 ± 833	433 ± 22
4	20	2.0	78.0	7.33	8.34	3.02 ± 0.05	76.3 ± 0.1	14041 ± 1102	408 ± 36
5	15	1.5	83.5	7.46	8.36	2.98 ± 0.01	75.5 ± 0.1	12791 ± 2402	408 ± 8
6	15	0.0	85.0	7.37	8.21	3.08 ± 0.05	75.6 ± 0.4	12583 ± 551	416 ± 22
7	10	1.0	89.0	7.67	8.45	3.08 ± 0.02	74.3 ± 0.1	18416 ± 6981	483 ± 46
8	5	0.5	94.5	7.90	8.55	3.05 ± 0.02	73.1 ± 0.1	13000 ± 1443	491 ± 44
9	0	3.0	97.0	8.39	8.60	2.90 ± 0.06	72.4 ± 0.3	15083 ± 2402	391 ± 8
10	0	1.5	98.5	8.27	8.51	2.98 ± 0.03	71.9 ± 0.3	12375 ± 2194	383 ± 58
11	0	0.0	100.0	8.15	8.57	3.09 ± 0.07	71.9 ± 0.2	12166 ± 908	483 ± 58

Table 3. Modified Gompertz model parameters determined from the experimental data.

Substrates	BMP [mL CH ₄ g ⁻¹ VS _{initial}]	μ_m mean [mL CH ₄ g ⁻¹ VS d ⁻¹]	λ mean [d]	R ²
M30 G3.0 CL67.0	131.1 ^a	3.30 ^a	17.36 ^a	0.9986 0.9943 0.9967
M30 G0.0 CL70.0	129.6 ^a	2.84 ^{a,b}	18.83 ^a	0.9920 0.9937 0.9945
M25 G2.5 CL72.5	127.7 ^{ab}	3.02 ^{a,b}	19.86 ^a	0.9953 0.9923 0.9963
M20 G2.0 CL78.0	116.5 ^{ab}	2.77 ^{a,b}	15.36 ^a	0.9937 0.9886 0.9932
M15 G1.5 CL83.5	122.3 ^{ab}	2.90 ^{a,b}	20.32 ^a	0.9832 0.9897 0.9923
M15 G0.0 CL85.0	117.8 ^{ab}	2.62 ^{a,b}	18.79 ^a	0.9885 0.9905 0.9917
M10 G1.0 CL89.0	118.3 ^{ab}	2.23 ^b	14.16 ^a	0.9896 0.9900 0.9945
M5 G0.5 CL94.5	115.5 ^{ab}	2.55 ^{a,b}	18.0386 ^a	0.9847 0.9962 0.9934
M0 G3.0 CL97.0	116.7 ^{ab}	2.39 ^{a,b}	21.7339 ^a	0.9898 0.9784 0.9802
M0 G1.5 CL98.5	97.4 ^b	2.44 ^{a,b}	26.4479 ^a	0.9741 0.9900 0.9772
M0 G0.0 CL100	113.2 ^{ab}	2.83 ^{a,b}	14.1252 ^a	0.9939 0.9942 0.9870

Table 4. Chemical composition of digestate after 90 days.

No.	pH	TS [% D.B.]	VS/TS [%]	COD [mg L ⁻¹]	N-NH ₃ [mg L ⁻¹]
1	7.48	1.83 ± 0.18	60.0 ± 2.7	1533 ± 136	715 ± 32
2	7.51	1.92 ± 0.09	67.5 ± 1.3	1551 ± 28	776 ± 27
3	7.33	1.50 ± 0.19	58.7 ± 2.9	1542 ± 16	666 ± 1
4	7.37	1.72 ± 0.12	58.7 ± 1.6	1296 ± 116	630 ± 71
5	7.36	1.73 ± 0.04	58.9 ± 1.9	1375 ± 106	706 ± 54
6	7.46	1.57 ± 0.04	62.4 ± 0.4	1500 ± 25	718 ± 4
7	7.44	1.90 ± 0.22	56.5 ± 1.5	1278 ± 52	560 ± 12
8	7.40	1.77 ± 0.15	60.4 ± 0.5	1185 ± 130	551 ± 11
9	7.48	1.57 ± 0.04	56.9 ± 1.5	1292 ± 135	508 ± 7
10	7.51	1.52 ± 0.01	58.9 ± 2.8	1227 ± 58	565 ± 5
11	7.50	1.62 ± 0.09	55.8 ± 2.8	1153 ± 82	576 ± 10