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Ecofriendly manure anaerobic digestion assisted by soluble bio-based substances obtained from anaerobic digestion, composting and chemical hydrolysis of urban bio-wastes. A step toward the integration of urban and agriculture waste management

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Ecofriendly manure anaerobic digestion assisted by soluble bio-based substances obtained from anaerobic digestion, composting and chemical hydrolysis of urban bio-wastes. A step forward the integration of urban and agriculture waste managements

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Abstract

BACKGROUND: recently, a virtuous material cycle has been reported where soluble bio-based substances (SBO) obtained by anaerobic digestion, composting and chemical hydrolysis of urban wastes are recycled at 0.05-2 % concentration to the anaerobic bioreactor to reduce the ammonia content in the fermentation digestate, with no adverse effect on biogas yield and composition. Scope of the present work was to assess whether the same SBO effect was real also for the anaerobic fermentation of farm manure.

RESULTS: manure fermentations were carried out at 35 °C for 40 days in the absence (control) and in the presence of 0.2 % SBO using a 128 liter reactor, yielding over 1000 biogas NL. No SBO effect on biogas production and composition was evident. The control fermentation produced 18 % ammonia at the expense of the pristine organic N. On the contrary, the fermentation in the presence of SBO produced no ammonia and no reduction of the pristine organic N.

CONCLUSIONS: the SBO assisted anaerobic fermentation for reducing the digestate ammonia content can be applied to farm manure as well as to urban biowastes. This finding prospects more benefits from a wider material cycle including both urban and agriculture wastes processed by integrated chemical and biochemical technology.

Keywords: anaerobic and aerobic fermentation; hydrolysis; ammonia abatement; manure; added value bio-based products

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Introduction

Anaerobic digestion systems can be located either on-farm, or at larger centralized anaerobic digestion management facilities.[1] A large part of farm biogas plants run on animal excrement.[2] These allow converting farm manure to biogas and use the product as fuel for the local energy needs. However, the process yields a digestate containing the recalcitrant organic matter not converted to biogas, and ammonia produced by action of proteolytic bacteria on the pristine protein matter present in manure. The presence of ammonia in the digestate poses the problem of its reuse or disposal. In principle, the digestate is rich in soil nutrients and might be recycled to farmland. However, ammonia emission and/or nitrate leaching can occur due to inappropriate handling, storage and application of digestate as fertilizer.[3] In Europe, the Nitrate Directive (91/676/EEC) restricts the input of mineral nitrogen on farmland, aiming to protect the ground and surface water from pollution. Downstream technology is available for removing excess inorganic N from the digestate.[4, 5] This however requires additional substantial process costs. The abatement of ammonia from the digestate through the biochemical Anammox process can run 1.6 \$ kg^{-1} nitrogen.[6] Other authors[7] report cost estimates running from 1 to 13 \$ kg^{-1} N for ammonia recovery by the available physico-chemical technologies, such as membrane separation, air stripping, ion exchange, chemical precipitation. In addition, the facilities for the secondary treatment of the digestate are available for large centralized biowastes management installations, require a high capital cost, and are not economically sustainable at farm level. So far, a viable solution for properly managing the manure digestate at farm level is not available. The problem is rather challenging, considering the following figures. As of 2012, the entire manure production in the EU that is potentially available for processing was estimated 1.4 billion tonnes.[8] This production results from a myriad of farms spread over large areas.[9] Only about 8 % of the manure production, equal to 108 million ton, containing 556000 ton nitrogen, is currently being processed by thousands of installations throughout EU member states. It is obvious that these circumstances require a simple economically sustainable solution to the problem of ammonia production in the anaerobic fermentation of manure. This

solution should be applied locally, in on-farm installations of any size, thus avoiding collection and transportation costs of the digestate to larger centralized plants for secondary treatment.

Most recently,[10] it has been reported that ammonia in the anaerobic digestate of municipal biowastes can be reduced, without adversely affecting biogas yield and quality, by simply adding 0.05-0.20 % of biowaste derived soluble bio-based substances (SBO) to the anaerobic fermentation feed. This finding prospects that anaerobic digestion in the presence of SBO does not require secondary treatment of the digestate for reducing the ammonia content, and that therefore no capital cost for digestate processing facilities is necessary. Scope of the present work was therefore to assess whether the SBO effect on reducing the digestate ammonia was real also for the anaerobic fermentation of manure.

The authors' interest in the SBO was cultural, broader than just the scientific value of the effects in the anaerobic fermentation of manure. The SBO products are isolated from the alkaline hydrolysate of composted municipal biowaste (MBW).[11] They contain a mix of polymeric molecules constituted by aliphatic and aromatic C bonded to several different acid and basic functional groups, which in turn complex several mineral elements. These chemical features represent the memories of the protein, fats, polysaccharide, and lignin proximates constituting the pristine biowaste. They are associated to the SBO properties as surfactants, agents for sequestering or carrying small molecules and mineral ions in solution, photosensitizers and reactive biopolymers. Particularly interesting is previous work reporting the performance of SBO as plant growth biostimulants,[12] biopolymers for the fabrication of mulch fims[13] for agriculture use, and animal feed supplement.[14, 15] In this context, assessing the SBO performance in reducing ammonia in the digestate obtained from farm manure fermentation represented a further demonstration that integrated chemical and biochemical technology could effectively realize a virtuous, cost-effective, environmentally friendly and socially favored renewable organic C cycle involving urban and farm biomass and wastes.

EXPERIMENTAL METHODOLOGY

Fermentation liquor and SBO preparation

The fermentation liquor was collected from Fontanacervo farm in Villastellone (TO), Italy. It consisted in the as collected farm cow manure and the digestate recovered from the manure biogas production facility, as inoculum. The preparation and chemical characterization of SBO was accomplished according to previous work.[11] The product was obtained by alkaline hydrolysis in water at pH 13 and 60 °C of composted urban private gardening residues and public park trimmings aged for 110 days, as reported elsewhere.[11] The hydrolysate slurry was centrifuged to separate the solid form the liquid phase. The latter was filtered through a 5 kD cut-off polysulphone membrane, and the membrane retentate was dried to yield the solid SBO. The product chemical composition (Table 1) was obtained by C and N microanalysis, 13C NMR spectroscopy, potentiometric titration, volatile solids determination, and ICP atomic emission spectrometry.

Set up of anaerobic digestion trials.

The biogas trials were performed with a previously described reactor.[16] This is a 316 stainless steel cylindrical tank 90 cm high, with a diameter of 40.3 cm. It is closed by two caps on the top and on the bottom. The total reactor volume is128 liters. The working volume is about 100 liters. It is equipped with mechanical stirrer, automatic measuring and recording of the biogas cumulative volume, temperature and pH. The stirring system consists in a blade propeller and a scraper on the bottom, both made by 316 stainless steel. The stirring speed is controlled through variable speed electric engine that is controlled by an inverter.

The digester and the gasometer were equipped with a complete probe monitoring system including a temperature probe inserted on one side of the reactor, a temperature and a pressure probe on the gas holder, a pH probe inserted inside the digester. The temperature was maintained near 35 °C by mean of an electrical resistance (15 m) was wrapped around the reactor and covered

with insulating film. The system was equipped with a small tank designed for condensing the vapor produced in the reactor and being emptied automatically. The produced biogas cumulative volume was measured by mean of a slide-wired potentiometer, which was linked from one side with the gasometer upper parts and fixed with the chassis from the other. The operational pressure was about 9-10 mbar. The outlet pipe was equipped with a solenoid valve activated by a relay to allow the automatic discharge of the biogas. The reactor was filled up with 72 liters of farm cow manure and 8 liters of inoculum, flushed with nitrogen, and kept at 35 °C for 40 days, when the biogas production was substantially negligible. Under these experimental conditions, six fermentation runs were carried out, namely runs 1 through 6 at the following starting dates: run 1 (control) on November 28, 2013, run 2 (treatment) on January 10, 2014, run 3 (control) on April 10, 2014, run 4 (treatment) on June 10, 2014, run 5 (control) on July 15, 2015, run 6 (treatment) on September 10, 2015. Each run was carried out with manure and inoculum collected at the sourcing farm the day before the run start day. The experiments were designed to account for the yearly seasonal variability of the collected materials. The amount of SBO added in the treatment runs was 160 g, corresponding to 0.2 % SBO concentration in the starting fermentation slurry feed. Prior to the fermentation turns, the SBO biogas potential was measured according to literature.[17] It resulted 15.8 □ 0.33 NL/kg SBO.

Reactors mass balance and analytical procedure

The reactor was charged with a previously weighed amount of fermentation feed liquor. At the end of the reactions, the total content of the reactor was discharged and weighed. Triplicates analysis was performed on samples withdrawn from the starting and the final liquor of each reactor. The sample was centrifuged. The separated liquid and solid phase were weighed to determine their relative content in the reactor liquor. They were analyzed separately for the content of moisture, total solids (TS), volatile solids (VS), ashes, total N (N_{tot}), ammonia N (N_{NH3}) and organic N (N_{org}), according to previously reported methods.[10] This procedure allowed calculating the total amount of

the above analytes in each reactor at the beginning and at the end of the fermentation run. The reactor gas phase was analyzed recording continuously the biogas cumulative volume as a function of the reaction time. The gas phase was sampled at time intervals and analyzed for its content of CH_4, CO_2, N_2, O_2, H_2, and H_2S by GA-2000 gas analyzer.

Statistical analysis of the experimental data

Unless otherwise stated, all data are expressed as mean ± standard error. When possible the means of all the parameters were examined for significance by single factor Analysis of Variance (ANOVA) using the software JMP version 9 (SAS Institute Inc, Cary, North Carolina, USA). When F values showed significance, individual means were compared using Tukey's honest significant difference at $P \leq 0.01$. The experimental biogas volume versus the fermentation time were fitted with the following modified Gompertz model,[10]

$$Y_t = y_{max} \cdot \left\{1 - exp\left[-\frac{A}{y_{max}}(t - t_0)\right]\right\} \quad (1).$$

where t is time (days), Y_t is the cumulative biogas production volume (NL) at time t, y_{max} is the total biogas volume produced at fermentation end (NL), A is the maximum methane production rate in the log phase (NL/day) and t_0 represents the lag phase (days).

RESULTS AND DISCUSSION

Composition of SBO and basic fermentation slurry

The SBO chemical data, which are reported in Table 1, shows that the product contains both organic and mineral matter. The former is constituted by organic macromolecules containing aliphatic and aromatic C bonded to several acid and basic functional groups. The weight (MW) and number (MN) average molecular weights, and the MW/MN DI ratio show that a mix of molecules with different molecular weight composes the SBO. It is also likely that the C types and functional

groups listed in Table 1 were not homogeneously distributed over the macromolecular pool. The mineral fraction of the products contains several main and trace elements, presumably bonded to the organic functional groups.

Table 2 reports the analytical data for manure and inoculum used in the control and in the treatment runs. The data accounts for the seasonal variability of the used materials sampled from November 2014 through September 2015. Generally, the materials used in the control and in the treated runs do not appear significantly or much different.

Biogas production

The biogas cumulative versus fermentation time plots for the control and treatment runs exhibited similar trends. They were similar to that characterizing the bacterial growth, showing the typical lag, log and stationary phases. Figure 1 shows a typical plot. During the fermentation time, the pH was always inside a range of 6.8 to 7.7. The lag phase was very short, 0-3 days. The log phase lasted about 15 days. After this time, the gas production rate decreased about 60% and the curves flattened out as the gas production approached the stationary phase. Table 3 reports the measured total biogas volume at fermentation end and the results of fitting the experimental data for the exponential phase. The reported values are averages calculated separately over the three control runs and the three treatment runs (see Set up of anaerobic digestion trials subsection). The data allows the following observations.

The biogas potential of the amount of SBO added to the fermentation slurry feed (see Experimental Methodology) allows calculating 2.5 NL biogas production over the duration of each fermentation run. The rather low biogas potential of the SBO was expected. This material is the soluble hydrolysate of mature compost (see Experimental methodology). Its chemical composition (Table 1) contains the memory of the recalcitrant aromatic lignin proximate present in the pristine biowaste sourcing material. The product can be recovered upon acidification of the fermentation digestate in high yields, relatively to the added amounts at the start of fermentation. In the dry state,

the SBO is stable up to 200 °C.[13] It can be melt extruded at this temperature to yield poly(ethylene-co-vinyl alcohol) composite films.[17] It can be oxidized only by strong reagents. A most recent work[18] reports that the ozonation of SBO opens the aromatic lignin moieties yielding poly hydroxyl carboxylic aliphatic moieties, but negligible mineralization. Thus, the SBO biochemical, chemical and thermal stability is relatively high.

In the fermentation runs of the present work, the potential 2.5 NL biogas contribution by SBO is rather negligible compared to the 1028-1111 NL average cumulative volumes produced in the control and treatment runs. The data in Table 3 shows that there is no significant effect on the biogas production by the addition of SBO to the fermentation slurry. Moreover, no significant SBO effects were observed on the biogas composition. Table 4 reports typical data for the control run 1 and the treatment run 2. It may be observed that in both cases CO_2 and CH_4 account for nearly 100 % of the gas phase, and there is no significant difference in the CH_4/CO_2 ratio of the two runs.

Condensed phase analysis

Table 5 reports the chemical analytical data of the liquid phase at the start and end of the fermentation for the four runs. The most meaningful values are the analytes' % changes at fermentation end, relative to the starting value, which are observed reported for volatile solids and ammonia. The data shows that the VS (i.e. the organic matter) consumption in the treatment runs is 16 % lower in the control runs. However, this lower organic matter consumption is not reflected in a significantly different biogas production, as shown by Table 3 data. The nitrogen balance data shows that, both in the control and in the treatment runs, there is no significant total N % change. This means that, both in the control and in the fermentation runs, the total N at fermentation start is recovered quantitatively at fermentation end. Thus, there is no significant nitrogen loss in the gas phase. The result is consistent with the gas phase composition data in Table 4, showing essentially no N_2 in the gas phase, and CO_2 and CH_4 accounting for over 99 % of the total gas phase composition. The no N loss in the gas phase is further supported by the values reported for total

nitrogen weight in the starting fermentation slurry and in the recovered slurry at fermentation end. These figures show that, both in the control and fermentation runs, the total N is quantitatively accounted by the weights of ammonia and organic N. However, mostly interesting is that the fact that the relative distribution of ammonia and organic N in the control and in the fermentation runs are different. Specifically, the % change values show that the control fermentations produce ammonia (% change 18.2) at the expense of organic N (% change – 25 %). On the contrary, in the treatment fermentation runs, no ammonia is produced and no organic N is consumed.

Relevance of the results

Consistently with previous data obtained on the effect of SBO in the anaerobic digestion of urban kitchen wastes,[10] the data obtained in this work confirm that SBO are efficient auxiliaries to obtain also manure digestates with low ammonia content. The full relevance of these results can be appreciated in connection with the other SBO properties as plant growth biostimulants,[12] biopolymers for the fabrication of mulch fims[13] for agriculture use, and animal feed supplement.[15] All together, these properties propose SBO as a multipurpose efficient interphase between urban and agriculture environments. Applying SBO to farm soil means returning renewable C to promote production of biomass for human consumption, which will generate more urban bio-waste, from which other SBO may be produced through integrated chemical and biochemical technology. The use of SBO in agriculture is not only limited to plant growth. These substances may return renewable organic C to cultivation soil in form of mulch film or remain included in ecofriendly animal manure. Indeed, the effect of SBO to reduce the ammonia content in manure anaerobic digestate is effective not only in the fermentation performed in dedicated farms bioreactor, but also in the feed digestion occurring in the animal intestine. In vitro[14] and in vivo[15] animal studies have demonstrated that adding 0.1 % SBO to swine and rabbits animal feed reduces the ammonia content in the animal feces, with no negative effect on animal growth and health. Thus, in farms, the SBO effect in reducing the manure ammonia environmental impact may be exploited in two ways,

i.e. (i) by adding SBO in the animal feed to produce manure with low ammonia content, and (ii) by adding SBO to the dedicated manure anaerobic digester to produce digestate with low ammonia content, for further use as fertilizer.

CONCLUSIONS

This work proposes a new ecofriendly SBO assisted process for the anaerobic fermentation of manure. It demonstrates that soluble substances (SBO), obtained from municipal biowastes through integrated chemical and biochemical technology,[11] and added at low concentration (0.20%) to manure slurry, allow producing anaerobic digestate with low ammonia content. The same SBO effect had been previously observed for the anaerobic digestion of humid organic fraction of solid municipal biowastes.[10] The results reported now widen the range of applications of the SBO. They affect the management of both urban and agriculture wastes. In the case of manure, they prospect a simple economically sustainable solution to the problem of ammonia production. This solution can be applied locally, in on-farm anaerobic digestion installations of any size, thus avoiding collection and transportation costs of the digestate to larger centralized plants for secondary treatment.

Moreover, the results of the present work widen the number of potential applications for the SBO, and the perspectives of their marketability. This fact may contribute to convince investors to allocate capital investment funds in SBO production facilities. The SBO production should occur into large size urban biowaste plants, which could then supply the needed product amounts to farmers. The installation of the biowaste chemical hydrolysis facility into municipal biowaste treatment plants producing biogas would benefit from the availability of the on-site collected biowaste and produced biogas to cover the energy needs of the chemical process. This scenario would allow integrating chemical technology for the production of SBO with current conventional anaerobic and aerobic fermentation processes.[10,11] It could be the first step to validate a biorefinery fed with municipal biowastes to produce value-added products for diversified uses. It depicts also an intriguing renewable C cycle taking place over agriculture and urban wastes through the SBO.

The potential environmental and economic benefits at stake, from the SBO assisted anaerobic digestion process, coupled to the other uses of SBO in the chemical, agriculture and animal feed supplement sectors, are high. They justify further worthwhile investigation to assess the full potential of the municipal biowaste derived SBO to cover needs stemming from agriculture and human product consumption.

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Table 1. Analytical data[a] for SBO.

pH	Ashes (w/w %)	C (w/w %)	N (w/w %)	C/N
8.2± 0.1	27.9 ± 0.6	38.25 ± 0.09	4.01 ± 0.03	9.54

Mineral elements: Si, Fe, Al, Mg, Ca, K, Na (w/w%); Cu, Ni, Zn, Cr, Pb, Hg (ppm)												
Si	Fe	Al	Mg	Ca	K	Na	Cu	Ni	Zn	Cr	Pb	Hg
2.55 ±0.01	0.77 ±0.04	0.49 ±0.04	1.13 ±0.06	6.07 ±0.38	3.59 ±0.21	0.16 ±0.01	202 ±4	92 ±1	256 ±1	19 ±1	85 ±1	0.15 ±0.02

C types and functional groups[b]concentration as mole fraction of total organic C, and molecular weight data[c]												
Af	NR	OMe	OR	OCO	Ph	PhOH	PhOY	COOH	CON	C=O	MW	DI
0.37	0.07	0.00	0.14	0.04	0.13	0.05	0.02	0.12	0.01	0.05	79.6	3.4

[a]Concentration values referred to dry matter: averages and standard deviation calculated over triplicates; [b]relative standard deviations were found to be within 10 % of mean values for aliphatic (Af), aromatic (Ph), methoxy (OMe), amide (CON), ammine (NR), alkoxy (RO), phenoxy (PhOY), anomeric (OCO), carboxylic acid (COOH), phenol (PhOH) and ketone (C=O) C. [c]Weight (MW) and number (MN) average molecular weight (MN), and dispersity index DI = MW/MN.

Table 2. Analytical data for as collected manure slurry and inoculum used to make up the basic feed fermentation slurries for the control (CTR) runs 1,3,5 and for the treatment (TRM) runs 2.4.6: total solids (TS), volatile solids (VS), ashes, and total (N_{tot}), ammonia (N_{NH3}) and organic (N_{org}) nitrogen % w/w concentration, relative to dry matter, and SV/N_{tot} ratio.[a]

				TS	SV	N_{tot}	N_{NH3}	N_{org}	SV/N_{tot}
CTR			Manure	5.53±0.028 (A)	4.338±0.025 (A)	0.281±0.004 (A)	0.140±0.0 (A)	0.141±0.004 (A)	15.46±0. (A)
			Inoculum	5.535±0.035 (b)	3.595±0.022 (b)	0.362±0.004 (a)	0.169±0.002 (a)	0.193±0.002 (a)	9.93±0. (b)
TRM			Manure	5.653±0.004 (A)	4.253±0.005 (A)	0.23±0.014 (A)	0.1±0.0 (B)	0.13±0.014 (A)	18.54±1. (A)
			Inoculum	5.79±0.014 (a)	3.905±0.021 (a)	0.370±0.0 (a)	0.187±0.004 (a)	0.183±0.004 (a)	10.55±0. (a)

[a]Average values calculated over runs 1,3,5 for the control (CTR) and over runs 2,4,6 for the treatment (TRM) fermentations. Within each column, values followed by different capital (A) and (B) letters, and values followed by different lower case (a) and (b) letters are significantly different at $P \leq 0.01$ level.

Table 3. Measured total biogas volume at fermentation end and results of fitting experimental data for the exponential phase with eq 1. [a] Values are averages calculated over runs 1,3,5 for the control and over runs 2,4,6 for the treatment fermentations.

Parameters	Control	Treatment
Measured biogas total volume at fermentation end (V_{mt}, NL)	1111±334	1028±174
y_{max} (NL)	1201±325	1165 ±350
A (NL day^{-1})	70.7 ±25.7	75.5 ±12.6
t_0 (day)	1.17 ±0.89	1.65 ±0.77
R^2	0.994±0.002	0.987±0.009

[a] y_{max}, extrapolated total biogas volume at the end of the exponential phase; *A*, exponential phase gas production rate; t_0, lag phase time; R^2, correlation coefficient. Within each row, values are not significantly different at $P \leq 0.01$ level.

Table 4. Experimental values for biogas composition (% or ppm mole/mole) as a function of fermentation time, and mean and standard deviation (Std) values calculated over the total fermentation time.[a] Total % (7th column) = sum of all analyzed gas molecules %.

RUN N° 1

Days	*CH4 %*	*CO2 %*	*N %*	*CO ppm*	*H2S ppm*	*Total*	*Tot Carbon (CH4+CO2)*	*CH4/CO2*
0								
2	54.9	38.8	0.2	6.1	466	93.9	93.7	1.41
5	66.5	34.2	0	0		100.7	100.7	1.94
7	58.4	41.7	0.3	0	728	100.4	100.1	1.4
9	54	45.7	0	0.3	983	99.7	99.7	1.18
16	54	45.6	0	0.4	692	99.6	99.6	1.18
19	53.8	45.7	0	0.5	683	99.5	99.5	1.17
21	54	46.2	0	0	633	100.2	100.2	1.16
23	52	48	0	0	831	100	100	1.08
26	53.7	45.9	0	0.4	829	99.6	99.6	1.16
28	54.3	45.6	0	0.1	837	99.9	99.9	1.19
30	55.2	44.3	0	0.5	783	99.5	99.5	1.24
36	54.5	45.1	0	0.4	723	99.6	99.6	1.2
40	56	43.7	0	0.3	767	99.7	99.7	1.28
Mean	55.48 A	43.88 ab	0.03 A	0.69 a	727.3 A	99.40 a	99.36 A	1.28 a
Std	3.62	3.7	0.09	1.63	140.01	1.69	1.73	0.22

RUN N° 2

Days	*CH4 %*	*CO2 %*	*N %*	*CO ppm*	*H2S ppm*	*Total*	*Tot Carbon (CH4+CO2)*	*CH4/CO2*
0	26.6	68.09	0.2	5.09		94.89	94.69	0.39
2	30.2	66.59	0	3.2		96.79	96.79	0.45
4	51	48.1	0	0.9		99.1	99.1	1.06
7	74.09	26.9	0	0	865	101	101	2.75
9	63.1	37.4	0	0	748	100.5	100.5	1.68
11	63.2	37.4	0	0	624	100.6	100.6	1.68
18	58.9	41	0	0	450	99.9	99.9	1.43
21	56.8	42.9	0	0.3	417	99.7	99.7	1.32
24	56.5	43.3	0	0.2	356	99.8	99.8	1.3
28	56.5	43.2	0	0.3	388	99.7	99.7	1.3
30	56.3	43.6	0	0.1	382	99.9	99.9	1.29
32	56	43.8	0	0.2	372	99.8	99.8	1.27
35	55.8	44.4	0	0	312	100.2	100.2	1.25
37	55.7	44.2	0	0.1	447	99.9	99.9	1.26
42	55.4	44.4	0	0.2	299	99.8	99.8	1.24
Mean	54.4 A	45.02 a	0.01 A	0.70 a	577.33 A	99.44 a	99.42 A	1.31 a
Std	11.82	10.29	0.05	1.46	270.17	1.56	1.6	0.53

[a]For each analyte, control and treatment mean values do not differ significantly at $P \leq 0.01$ level.

Table 5. Process material balance: dry matter weight (g) of total solids (TS), volatile solids (VS), and total (N_{tot}), ammonia (N_{NH3}) and organic (N_{org}) nitrogen fermentation start and end, and % change of values at fermentation end, relative to values at fermentation start.[a] Values are averages calculated over runs 1,3,5 for the control (CTR) and runs 2,4,6 for the treatment (TRM) fermentations.

Run		TS	SV	N_{tot}	N_{amm}	N_{org}
CTR	Start	4.42 ± 0.02(a)	3.41 ± 0.02(a)	0.23 ± 0.00(a)	0.11 ± 0.00(a)	0.12 ± 0.00(a)
	End	2.67 ± 0.04(B)	1.82 ± 0.03(B)	0.22 ± 0.00(A)	0.13 ± 0.00(A)	0.09 ± 0.00(A)
	% Change[a]	-39.6 ± 0.69(c)	-46.6 ± 0.6(c)	-4.34 ± 2.13(d)	18.2 ± 0.7(d)	-25 ± 3(d)
TRM	Start	4.53 ± 0.00(a)	3.37 ± 0.00(a)	0.19 ± 0.01(a)	0.09 ± 0.00(b)	0.10 ± 0.01(a)
	End	3.49 ± 0.01(A)	2.05 ± 0.01(A)	0.19 ± 0.01(A)	0.09 ± 0.00(B)	0.10 ± 0.01(A)
	% Change[a]	-23.0 ± 0.1(d)	-39.2± 0.2(d)	0.0 ± 8.2(d)	0.0 ± 3.5©	0.00 ± 0.63(c)

[a] % change = 100 (value at fermentation end – value at fermentation start)/value at fermentation start; in each columns, start values, end values, and % change values couples followed by different (a) and (b), (A) and (B), and (c) and (d) letters, respectively differ significantly at $P \leq 0.01$ level.

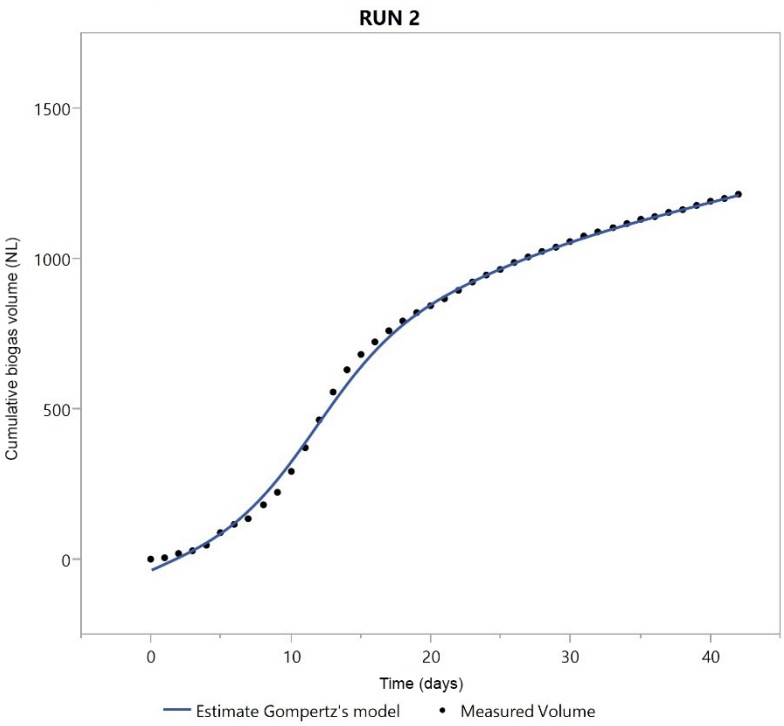

Figure 1. Experimental points and fitting curve according to eq 1 for biogas cumulative volume (NL) versus fermentation time (days) .