Grease trap waste valorization through hydrothermal liquefaction and anaerobic digestion: a circular approach to dairy wastewater treatment

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Analytical procedure details

The elemental composition (CHN) of GTW, biocrude, and hydrochar was determined using an Exeter Analytical CE-440 CHN/O Analyzer. The oxygen content was calculated by subtraction of the CHN and ash contents, with the latter measured by combustion of the sample at 550 °C to constant weight (APHA-2540E).[1] Total organic carbon (TOC) and total nitrogen (TN) concentrations (mg/L) in the AP were analyzed following the catalytic oxidation method at 720 °C using integrated Shimadzu TOC-L and TNM-L modules.
The organic constituents of the biocrude and the AP were identified by GC-MS, using an Agilent 6850 Series II GC coupled to an Agilent 5875B mass detector and an HP-Innowax capillary column. Compounds were identified by matching fragmentation patterns against NIST14 database.
The boiling point distribution of GTW and biocrudes was determined by thermogravimetric analysis (TGA) using a TA Instruments QA500.
The functional groups of hydrochars were characterized by fourier transform infrared (FTIR) analysis using a Thermo Scientific Nicolet iZ10 FTIR spectrometer.
The concentration of COD, Volatile fatty acids (VFAs), and total ammonia nitrogen (TAN), and pH were measured in the APs, BMP liquid phase, and influent and effluent of the EGSB reactor. To measure VFA concentrations, the liquids were acidified with 2% formic acid and measured by GC using a flame ionization detector (Agilent, USA) and a DB-FFAP capillary column (Agilent, USA). TAN was measured using an ion-selective electrode (HACH, USA) and pH was measured using a pH electrode (Thermo Electron Corporation, Singapore). Total organic nitrogen (TON) was estimated as the difference between TN and TAN, assuming that the presence of nitrates was negligible.

Table S1. Experimental design of HTL runs.

#	HTL Reaction	Reaction medium	Products		
1	R-0	DI	B-0	AP-0	HC-0
2	R-1	AP-0	B-1	AP-1	HC-1
3	R-2	AP-1	B-2	AP-2	HC-2
4	R-3	AP-2	B-3	AP-3	HC-3
5	R-4	AP-3	B-4	AP-4	HC-4
6	R-5	AP-4	B-5	AP-5	HC-5

Table S2. Composition of the nutrient media solution used in the BMP tests. Developed by Labatut et al.,[2] based on Owen et al.,[3] Angelidaki et al.,[4] and Speece et al.[5]

Basal medium	**Concentration in each BMP bottle (mg/L)**
NH_4Cl	200
KCl	100.0
$MgCl_2\ 6H_2O$	600.0
KH_2PO_4	138.0
K_2HPO_4	176.0
Vitamins	
Yeast extract	100
Trace elements	
$FeCl_3\ 6H_2O$	200.0
$MnCl_2\ 4H_2O$	4.0
$CoCl_2\ 6H_2O$	10.0
$NiCl_2 6H_2O$	10.0
$ZnCl_2\ 2H_2O$	0.5
Na_2SeO_3	0.1
$Na_2MoO_4\ 2H_2O$	0.5
$CaCl_2 2H_2O$	100.0
$CuCl_2\ 2H_2O$	0.5
KI	10.0
H_3BO_3	0.5
Others	
Resazurin	1.0
$Na_2S\ 9H_2O$	100.0
$NaHCO_3$*	4200

Table S3. Preparation of the anaerobic toxicity assay (ATA) for AP concentration volumes from 0 to 40%

	Control	**A (8%)**	**B (16%)**	**C (24%)**	**D (32%)**	**E (40%)**	**Blank**
AP concentration volume (%)	-	8	16	24	32	40	-
AP (mL)	0	4	8	12	16	20	0
Mass COD from AP (mg)	0	19	38	57	76	95	0
COD concentration from AP (g/L)	0	0.38	0.76	1.14	1.52	1.90	0
Standard feedstock (mL)	2	2	2	2	2	2	-
Inoculum (mL)	10	10	10	10	10	10	10
Nutrient solution (mL)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Distilled water (mL)	37.5	33.5	29.5	25.5	21.5	17.5	39.5
Final vol. (mL)	50	50	50	50	50	50	50
Inhibition (%)*	-	- 8%	- 11%	- 12%	5%	7%	-

*A negative value implies that the substrate at that concentration enhances biomethane production.

Table S4. Operational phases of the EGSB reactor

Phase	I	II	III	IV	V	VI	VII
Days	1 – 28	29 – 36	37 – 40	41 – 43	44 – 46	47 – 49	50 – 53
OLR (g COD L^{-1} d^{-1})	3	3	3	3	3	3	3
AP concentration (g COD L^{-1}) [a]	0.0	0.4	0.8	1.2	1.6	2.0	2.0
Synthetic substrate concentration (g COD L^{-1})	2.0	1.6	1.2	0.8	0.4	0.0	0.0
Total concentration (g COD L^{-1})	2.0	2.0	2.0	2.0	2.0	2.0	2.0

[a] AP-0 was used in Phases II to VI, while AP-R was used in Phase VII.

Table S5. Physicochemical characteristics of the grease trap grease

Proximate analysis (wt%)		Chemical composition analysis (wt%, daf)			Elemental analysis (wt%, daf)			
TS	Ash (db)	Crude fat [a]	Crude protein [a]	Total carbohydrate [b]	C	H	N	O [b]
48.06 ± 0.02	2.89 ± 0.13	76.60	9.43	13.97	67.2	11.0	3.3	15.6

Saturated fats [c] (wt%, daf)	Monounsaturated fats [c] (wt%, daf)	Poliunsaturated fats [c] (wt%, daf)
47.68	24.32	4.60

Inorganic composition (mg kg^{-1}, db) [d]

As	Cd	Cu	Sn	Fe	Pb	Se	Zn
< 0.08	< 0.02	5.6	< 0.1	556.3	5.9	< 0.05	16.8

TS: total solids; db: dry basis; daf: dry and ash-free basis.

[a] Fat and protein content analyses were performed at an external laboratory (Dictuc S.A., Chile), following the AOAC 989.05 and AOAC 2001.11 methods, respectively.
[b] Calculated by difference
[c] Lipid composition was performed at an external laboratory (Dictuc S.A., Chile), following the ISO 5509/ISO 15304 GC-FID FAME Analysis method
[d] Metals determination was carried out at an external laboratory (Dictuc S.A., Chile) according to Standard Methods 3030 C, E (2005) and Standard Methods 3120 B (2005). Preparation and digestion were based on AOAC 985.35 (2012).

Table S6. Major compounds identified* in the APs generated from the HTL of grease trap waste.

		Aqueous Phase (Area, %)					
RT (min)	Compound	**AP-0** [a]	**AP-1**	**AP-2**	**AP-3**	**AP-4**	**AP-5**
13.956	2,6-Lutidine	-	-	-	-	0.1	-
15.565	Pyridine, 3-methyl-	-	-	0.69	0.39	0.41	0.88
16.529	2-Propanone, 1-hydroxy-	0.2	0.3	0.29	-	-	0.3
16.829	Pyridine, 2,3-dimethyl-	-	-	-	-	0.2	-
16.838	1 Pyridine, 2,3-dimethyl-	-	-	-	0.2	-	-
17.095	1 Pyridine, 2,4,6-trimethyl-	-	-	-	0.25	-	-
17.104	Pyridine, 3,5-dimethyl-	-	-	-	-	-	0.19
17.514	Pyridine, 4-ethyl-	-	-	-	-	-	0.36
17.667	Benzenamine, 3,5-dimethyl-	0.18	-	-	-	-	-
17.711	Pyridine, 2,4,6-trimethyl-	-	0.4	0.26	-	0.72	0.28
17.960	Benzenamine, 2,4-dimethyl-	-	-	-	-	-	0.16
18.951	Acetic acid	21.52	11.89	14.92	14.61	15.6	13.08
19.527	Benzenamine, 2,4,6-trimethyl-	-	-	0.51	-	-	-
20.315	Formic acid	-	0.28	-	-	-	-
20.974	Propanoic acid	1.75	1.7	2.05	1.9	2.1	1.75
21.975	Benzenamine, 2-cyclopropyl-	-	-	0.2	-	-	-
22.994	Butanoic acid	2.63	3.2	3.66	3.63	3.91	2.55
23.266	Butyrolactone	-	-	0.78	-	-	-
23.294	Butanoic acid, 4-hydroxy-	-	0.45	-	0.44	0.67	0.48
25.449	Pentanoic acid	-	-	-	-	0.16	-
25.780	1H-Pyrrole, 2,3,4,5-tetramethyl-	0.15	1.06	0.41	0.21	0.46	0.52
26.174	Acetamide	-	-	-	0.3	0.43	-
26.847	Benzenamine, 4-methoxy-N-methyl-	-	0.08	-	-	-	-
27.047	1H-Pyrrole, 3-ethyl-2,4,5-trimethyl-	-	-	0.34	-	-	0.54
28.509	Piperidine, 1-methyl-	-	-	-	0.21	-	-
28.677	2,5-Pyrrolidinedione, 1-ethyl-	-	-	0.24	0.86	0.92	0.44
28.882	Butanamide	-	-	-	0.22	0.22	-
29.315	2,5-Pyrrolidinedione, 1-methyl-	0.71	-	1.31	-	1.94	-
29.861	Pyrrolidine, 1-methyl-	-	-	0.97	-	-	-
30.028	Quinoline	-	-	0.91	-	-	-
30.038	2-Propenenitrile, 3-phenyl-, (E)-	-	-	-	-	-	0.55
31.122	Phenol	-	0.26	-	0.35	-	-
-31.884	2-Pyrrolidinone	4.67	3.27	6.21	4.84	5.07	4.65
32.393	1,2-Propanediol	0.96	0.91	1.37	2.78	2.52	2.29
33.584	Quinoline, 4-methyl-	0.58	0.25	0.52	0.41	0.29	0.38
33.916	2-Piperidinone	0.97	0.34	0.72	1.69	-	-
34.679	L-Lactic acid	-	-	-	0.59	-	0.89

34.929	Caprolactam	0.3	0.23	0.32	0.4	-	0.23
35.874	2(1H)-Pyridinone, 1,3-dimethyl-	-	-	-	-	-	0.15
36.695	2(1H)-Pyridinone, 3,6-dimethyl-	0.63	-	-	1.73	1.4	0.32
36.797	3-Pyridinol, 2,6-dimethyl-	-	1.25	-	-	-	-
37.076	Glycerol	28.38	19.27	29.01	27.66	25.1	24.77
37.390	2-Ethyl-6-methylpyridin-3-ol	0.86	-	-	-	-	-
37.577	3-Pyridinol, 2-ethyl-6-methyl-	-	-	-	-	-	0.68
37.617	5-Dimethylaminopyrimidine	-	-	-	0.95	0.91	-
38.642	2(1H)-Pyridinone, 5-methyl-	2.5	3.06	-	4.39	0.31	-
38.754	3-Pyridinol, 6-methyl-	-	-	5.58	0.39	-	3.65
38.898	3-Pyridinol	1.45	1.35	1.97	-	1.71	1.74
38.930	4-Pyridinol	-	-	-	1.69	-	-
39.239	Phenol, 3-amino-	-	-	-	0.24	-	0.43
39.628	Succinimide	1.25	1.35	2.14	1.88	2.07	2.11
40.582	Phenol, o-amino-	0.36	0.23	0.28	-	3.42	0.44
40.979	Benzeneacetic acid	0.81	-	-	-	-	-
41.730	Isosorbide	0.33	0.38	0.47	0.46	0.39	0.4
43.686	3,6,9,12-Tetraoxatetradecan-1-ol	0.11	-	-	-	-	-
45.373	15-Crown-5	1.45	-	-	-	0.33	-
46.514	Hexaethylene glycol	-	1.12	-	-	-	-
46.802	18-crown-6	-	-	-	0.14	-	-
46.875	Hexaethylene glycol monododecyl ether	1.41	-	-	-	-	-
48.329	Benzeneethanol, 4-hydroxy-	2.51	4.06	1.26	0.77	1.5	1.27
48.517	Pentaethylene glycol	0.33	-	-	-	-	-
49.471	4-pyridinecarboxamide	0.65	-	-	-	-	-
49.550	9-crown-3	-	-	-	-	-	0.06

***** Compounds with match factor scores, between observed and reference mass spectrum, greater than or equal to 75% were assigned as identified compounds.

[a] The labeling rule is the “abbreviation of aqueous phase” + “recycle times”.

Table S7. Major compounds identified* in the biocrudes generated from the HTL of grease trap waste.

		Biocrude (Relative area, %)					
RT (min)	**Compound**	**B-0 [a]**	**B-1**	**B-2**	**B-3**	**B-4**	**B-5**
7.565	Pentanoic acid	0.11	-	-	-	-	-
9.477	Pentanoic acid, 4-methyl-	0.04	-	-	-	-	-
10.713	Hexanoic acid	0.4	0.66	0.75	0.91	1.14	0.87
16.985	Octanoic acid	3.53	4.8	4.23	4.65	5.14	3.59
22.688	n-Decanoic acid	5.6	5.91	6.01	6.37	6.37	4.52
23.168	Decanoic acid, ethyl ester	-	-	-	-	-	0.24
26.641	Dodecanoic acid, methyl ester	0.04	-	-	-	-	-
28.243	Dodecanoic acid	32.07	41.48	39.47	43.21	43.79	31.34
28.418	Undecanoic acid, ethyl ester	0.12	-	-	0.34	-	2.34
30.126	Tridecanoic acid	0.06	-	-	-	-	-
30.427	Decanoic acid, 2-propenyl ester	0.18	0.35	0.74	0.23		0.44
30.744	Dodecanoic acid, propyl ester	0.05	-	-	0.22	-	-
30.867	Cyclohexadecane	-	-	-	0.23	-	-
32.749	Tetradecanoic acid	12.3	-	-	-	-	-
33.142	Tetradecanoic acid, ethyl ester	0.06	-	-	-	-	-
34.08	Pentadecanoic acid	0.2	20.06	25.2	29.2	23.36	18.08
36.011	Hexadecanoic acid, methyl ester	0.16	0.28	-	-	0.27	-
37.285	n-Hexadecanoic acid	19.35	-	-	-	-	-
37.416	Hexadecanoic acid, ethyl ester	-	0.36	0.24	0.42	0.3	6.56
37.418	Pentadecanoic acid, ethyl ester	-	-	0.31	-	-	1.62
37.425	Hexadecanoic acid, 2-methyl-, methyl ester	2.69	-	-	-	-	-
39.09	Octadecanoic acid, 2-propenyl ester	0.66	2.87	2.39	0.77	2.09	2.95
39.332	Hexadecanoic acid, propyl ester	-	-	-	0.32	-	-
40.816	Undec-10-ynoic acid, decyl ester	-	-	0.34	-	-	-
40.826	(E)-9-Octadecenoic acid ethyl ester	-	-	-	0.6	-	-
41.084	Octadecanoic acid	1.09	-	-	-	-	-
41.31	Octadecanoic acid, ethyl ester	-	-	-	-	-	2.51
41.323	Docosanoic acid, ethyl ester	-	-	-	-	0.29	-
42.351	9-Octadecenoic acid	6.9	-	-	0.29	-	-
42.353	Erucic acid	-	-	-	-	0.41	-
42.381	Undec-10-ynoic acid, dodecyl ester	0.11	-	-	-	-	-
42.606	cis-9-Octadecenoic acid, propyl ester	-	-	-	0.22	-	-
44.182	2-Tridecenoic acid, (E)-	-	-	-	-	0.51	0.43
44.222	trans-2-Dodecenoic acid	0.17	0.52	0.6	0.39	-	-
45.961	Dodecanoic acid, cyclohexyl ester	0.07	-	-	-	-	-

46.278	Hexadecanoic acid, (2,2-dimethyl-1,3-dioxolan-4-yl)methyl ester	0.21	-	-	-	-	-
46.478	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	-	-	0.71	-	-	-
6.49	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	-	-	-	0.58	-	0.47
46.496	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	0.35	0.97	-	-	0.38	-
49.496	Octadecanoic acid, (2,2-dimethyl-1,3-dioxolan-4-yl)methyl ester	0.06	-	-	-	-	-
55.797	Dodecanoic acid, 1 ,2,3-propanetriyl ester	-	2.42	-	-	-	-

***** Compounds with match factor scores, between observed and reference mass spectrum, greater than or equal to 75% were assigned as identified compounds.

[a] The labeling rule is the “abbreviation of biocrude” + “recycle times”.

Table S8. Elemental analyses of the GTW and hydrochar samples obtained in this study (mean ± SD).

	C	H	N	Ash	O	HHV
GTW	67.2±0.7	11.0±0.2	3.3±0.5	2.9±0.1	15.6±0.5	34.7±0.5
HC-0	33.2±0.2	3.0±0.0	2.6±0.0	53.9±0.0	7.3±0.2	13.1±0.1
HC-1	31.8±0.5	2.9±0.1	2.6±0.1	53.8±4.7	9.0±0.3	12.9±0.2
HC-2	30.5±0.3	2.7±0.1	2.5±0.1	52.5±4.5	11.8±0.5	12.6±0.3
HC-3	31.2±0.4	2.6±0.1	2.2±0.1	51.5±0.6	12.5±0.5	12.7±0.3
HC-4	28.6±1.4	2.4±0.3	2.0±0.2	56.1±0.0	10.9±1.0	11.7±0.4
HC-5	29.9±0.3	2.6±0.0	2.1±0.0	52.7±3.9	12.7±0.2	12.2±0.2

Table S9. Physicochemical characterization of the BMP digestate after treating aqueous phases (mean ± SD).

	AP-0	AP-1	AP-2	AP-3	AP-4	AP-5
pH	8.22±0.04	8.21±0.03	8.22±0.03	8.22±0.02	8.24±0.04	8.23±0.05
TAN (mg N L^{-1})	251.5±0.5	233.1±2.2	279.3±4.5	269.0±6.2	339.0±1.0	368.7±15.9
Total VFA (mg COD L^{-1})	141.2±13.4	63.8±0.9	317.2±4.2	190.4±5.9	858.4±6.1	412.9±7.7
Acetate (mg COD L^{-1})	74.4±4.2	25.4±0.3	239.3±0.8	161.3±5.3	475.4±4.7	324.6±7.3
Propionate (mg COD L^{-1})	20.9±4.5	12.4±0.1	77.9±4.9	13.7±0.4	317.6±2.9	88.3±5.7
Isobutyrate (mg COD L^{-1})	10.6±4.6	4.0±0.2	ND	5.2±0.5	24.9±1.9	ND

TAN: total ammonia nitrogen; VFA: volatile fatty acids; ND: not detected

Table S10. Kinetic parameters of the modified Gompertz model for the different AP samples.

Aqueous Phase	AP-0 [a]	AP-1	AP-2	AP-3	AP-4	AP-5
P (mL/g COD)	223.3	225.5	245.1	234.0	156.3	196.3
Rm (mL/g COD/d)	17.9	13.7	11.7	9.4	8.2	8.2
λ (d)	8.3	5.2	8.4	7.1	9.3	9.2
R^2	0.995	0.998	0.997	0.994	0.989	0.994

P: maximum biogas production; Rm: maximum biomethane production rate; λ: lag time; R^2: coefficient of determination.
[a] The labeling rule is the abbreviation of "Aqueous Phase" + "recycle times".

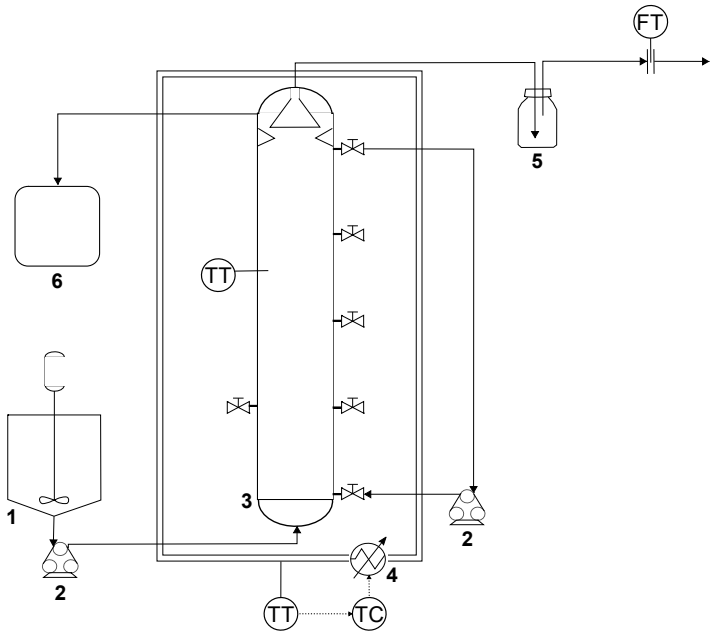

Figure S1. Diagram of the bench scale EGSB reactor used in this study

Substrate was constantly stirred and kept at 4 ^{o}C (1). A peristaltic pump (2) continuously fed the substrate to the reactor to maintain uniform flow.

The EGSB reactor (3) was made of polycarbonate with an inner diameter of 6 cm and a total height of 31 cm, height of digestion zone was 28.3 cm, having a capacity of 0.8 L. Three sampling ports were installed along the height of the reactor for taking samples for SMA test. The inlet was located at the bottom of the reactor. A holed piece was placed 5 cm from the bottom for dispersion of the inflow. A three-phase separator was located in the upper portion of the reactor for separating solid, liquid, and gas. Deflectors were projected horizontally, and the inverted funnel was fitted on the top of the reactor for biogas collection. The reactor was placed in a polycarbonate box equipped with 4 negative temperature coefficient (NTC) thermistors connected to a temperature controller (4). Additionally, there was one NTC thermistor installed inside the reactor to ensure a precise temperature of 37 ± 0.1 °C was maintained within the reactor. The biogas was passed through a CO_2 washing solution (5) and then to a gas counter (RITTER MilliGascounter type MGC-1) for the volumetric measurement of produced methane. The effluent was collected in the effluent tank (6).

The reactor was seeded with anaerobic granules from an internal circulation reactor that treats brewery wastewater, filling about 30% of the reactor volume. In the inoculum sludge, total solids, total suspended solids, and volatile suspended solids were 325.64, 312.04, and 301.80 g/L, respectively. The sludge was retained in the EGSB reactor during the experiment. The flow rate of the inflow was maintained at 1.193 L d^{-1} for the whole study, while the recirculation flow rate was kept to 24.225 L d^{-1}.

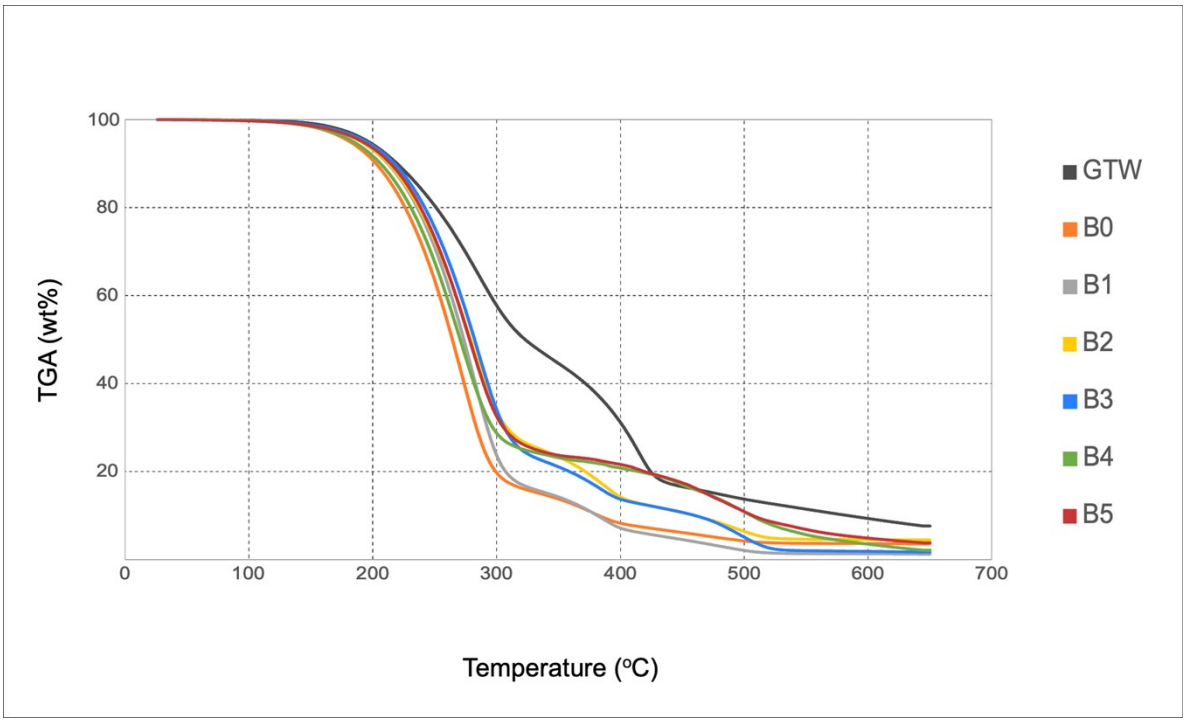

Figure S2. TGA analysis of the grease trap waste (GTW) and biocrudes. The labeling rule is the abbreviation of "biocrude" + "recycle times".

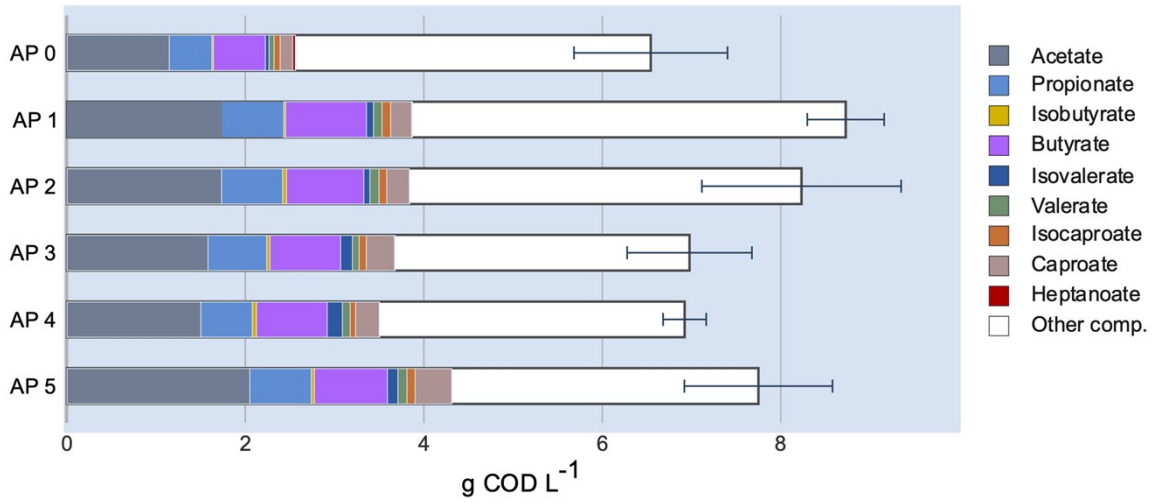

Fig. S3. Concentration of volatile fatty acids (g COD L^{-1}) characterized in the different APs. Bars are balanced with the total COD concentration of the sample.

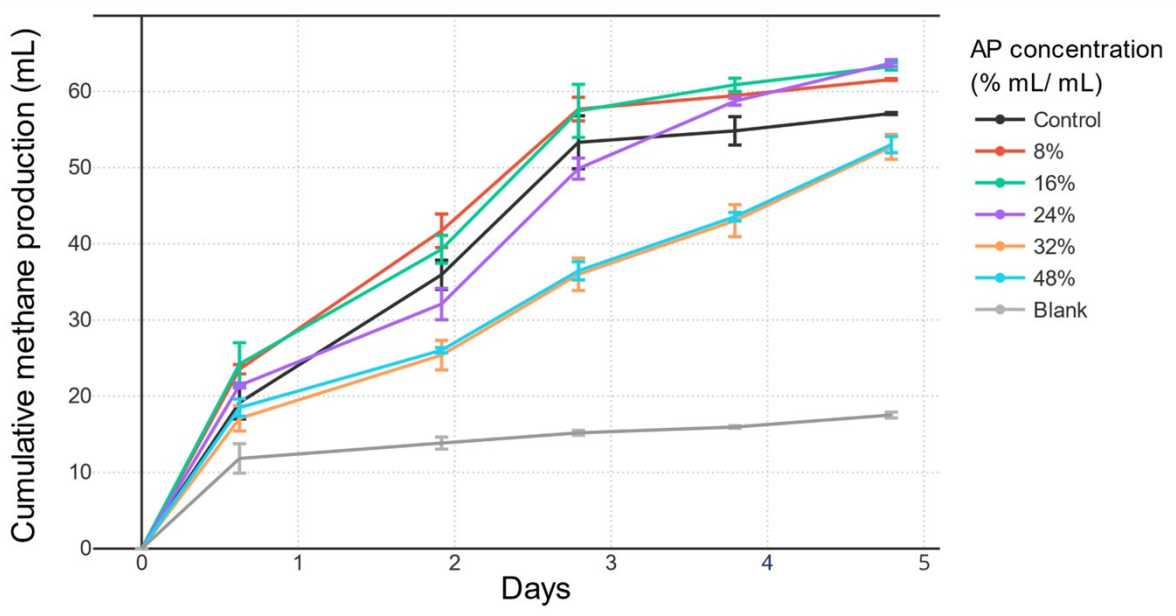

Figure S4. Cumulative methane production (mL) at increasing AP concentrations (i.e., 8, 16, 24, 32, and 48% v/v) following anaerobic toxicity assays (ATA).

References

1 APHA/AWWA/WEF, *Stand. Methods*, 2012, 541.

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