

1 **Electronic Supplementary Information**

2 **Characterizing the influence of wastewater composition and lignin content on** 3 **anaerobic biodegradability**

4

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21 column).

22 **Fig. S2.** Selected methane production curves (g CH₄-COD/g substrate-COD) as observed in the
23 BMP assays.

24 *Biomolecules*

25 The lab-grade biomolecules subjected to the assay were selected from each major chemical group,
26 to assess the effect of different physicochemical characteristics on organic substrate
27 biodegradability. Details on the chemical grade and other details of the pure biomolecules
28 provided by the lab suppliers are presented in Table S1.

29

30 **Table S1.** Lab-grade biomolecules subjected to the BMP assay.

Biomolecule	Supplier provided details	Supplier
Carbohydrates		
Cellulose	From cotton linters, Microcrystalline, 50 μm	Sigma Aldrich Co.
Xylan	From Beechwood, $\geq 90\%$ xylose residues	Sigma aldrich Co.
Starch	Powder, insoluble	B&A, Allied Chemical
D-Cellobiose	D(+), $>98\%$ purity	Acros Organics
D-Glucose	D(+), ACS	Sigma-Aldrich Co.
Proteins		
Casein	Pure	Acros Organics
Albumin	From eggs, powder	Acros Organics
Collagen	Insoluble	MP Biomedicals, LLC
Lipids		
Glycerol tripalmitate	Pure	TCI
Glycerol tristearate	Pure	TCI
Glycerol trioleate	Pure	TCI

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36 **Table S2.** Summary of BMP assays.

Lab-grade biomolecules	Lignocellulosic materials
<i>Carbohydrates</i>	○ Cow manure
○ Cellulose	○ Red onions
○ Hemicellulose, Xylan	○ Switchgrass
○ Starch	○ Wheat straw
○ Cellobiose	○ Miscanthus
○ Glucose	○ Corn silage
<i>Proteins</i>	
○ Casein	
○ Albumin	
○ Collagen	
<i>Lipids</i>	
Triglyceride blend (1:1:1 ratio VS basis):	
Glycerol tripalmitate (GTP), Glycerol	
tristearate (GTS), Glycerol trioleate	
(GTO)	

39 *Theoretical specific methane yields of biomolecules*

40 Table S3 presents the theoretical specific methane yields (B_u) of the biomolecules used in this
 41 study calculated using the Buswell Formula (Symons & Buswell, 1933).

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43 **Table S3.** Theoretical specific methane yields (B_u) and selected chemical properties of the biomolecules
 44 evaluated in this study.

Biomolecule	Bu at STP (L)		
	MWt (g/mol)	CH ₄ /kg VS)	COD (g COD/kg VS)
Carbohydrates			
Cellulose	162.1	414.5	1,184
Hemicellulose, Xylan	132.1	423.9	1,211
Starch	162.1	414.5	1,184
D(+)-Cellobiose	342.3	392.6	1,122
D(+)-Glucose	180.2	373.0	1,066
Proteins			
Casein	133.1	460.4	1,316
Albumin	134.4	462.7	1,322
Collagen	102.0	396.9	1,134
Lipids			
Glycerol tripalmitate (GTP)	807.3	1005.8	2,874
Glycerol tristearate (GTS)	891.5	1023.9	2,925
Glycerol trioleate (GTO)	885.4	1011.9	2,891

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46 The molecular formula of each protein was derived from its specific amino acid composition, as
 47 shown on Table S4. The amino acid composition of casein was obtained from the study of Ramsay
 48 and Pullammanappallil (2001), from which the resultant molecular formula was
 49 $C_{5.21}H_{9.92}O_{2.67}N_{1.21}S_{0.03}$. For egg albumin, the amino acid composition reported by Lewis et al.
 50 (1950) was used, and the derived molecular formula was $C_{5.24}H_{10.13}O_{2.62}N_{1.27}S_{0.05}$. Finally, the
 51 amino acid composition of collagen was obtained from Eastoe (1955) which resulted in the
 52 formula $C_{3.67}H_{7.49}O_{2.13}N_{1.16}S_{0.01}$.

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54

55 **Table S4.** Derivation of the empirical formulae for the proteins used in this study based on their amino
 56 acid composition.

57 Casein

Amino acid	(mol/c-mol)	n	a	b	c	d	MWt (g/mol)	Bu at STP (mL CH ₄ /g VS)	COD (g COD/g VS)
Isoleucine	0.0106	0.33	0.72	0.11	0.06	0.00	7.24	640.4	1.830
Leucine	0.0167	0.52	1.13	0.17	0.09	0.00	11.41	640.4	1.830
Lysine	0.0122	0.38	0.89	0.13	0.13	0.00	9.29	536.3	1.532
Methionine	0.0049	0.13	0.28	0.05	0.03	0.03	3.81	525.8	1.502
Phenylalanine	0.0078	0.37	0.45	0.08	0.04	0.00	6.71	678.0	1.937
Threonine	0.0075	0.16	0.35	0.12	0.04	0.00	4.65	376.1	1.075
Tryptophan	0.0016	0.09	0.10	0.02	0.02	0.00	1.70	630.7	1.802
Valine	0.0128	0.33	0.73	0.13	0.07	0.00	7.81	573.6	1.639
Arginine	0.0053	0.17	0.39	0.06	0.11	0.00	4.81	353.6	1.010
Histidine	0.005	0.16	0.23	0.05	0.08	0.00	4.04	360.9	1.031
Alanine	0.0076	0.12	0.28	0.08	0.04	0.00	3.53	377.1	1.078
Asparagine	0	0.00	0.00	0.00	0.00	0.00	0.00		0.000
Aspartate	0.0124	0.26	0.45	0.26	0.06	0.00	8.60	252.4	0.721
Cysteine	0.0003	0.00	0.01	0.00	0.00	0.00	0.19	314.3	0.898
Glutamate	0.0369	0.96	1.73	0.77	0.19	0.00	28.28	342.6	0.979
Glutamine	0	0.00	0.00	0.00	0.00	0.00	0.00		0.000
Glycine	0.0058	0.06	0.15	0.06	0.03	0.00	2.27	223.8	0.639
Proline	0.0217	0.57	1.02	0.23	0.11	0.00	13.01	535.0	1.529
Serine	0.0147	0.23	0.54	0.23	0.08	0.00	8.05	266.4	0.761
Tyrosine	0.0082	0.38	0.47	0.13	0.04	0.00	7.74	587.2	1.678
Hydroxyproline	0	0.00	0.00	0.00	0.00	0.00	0.00		0.000
Hydroxylysine	0	0.00	0.00	0.00	0.00	0.00	0.00		0.000
Sum	0.192	5.21	9.92	2.67	1.21	0.03	133.13	460.4	1.316

58

59 Egg albumin

Amino acid	g	n	a	b	c	d	MWt (g/mol)	Bu at STP (mL CH ₄ /g VS)	COD (g COD/g VS)
Isoleucine	7.1	0.40	0.86	0.13	0.07	0.00	8.68	640.4	1.830
Leucine	9.9	0.55	1.20	0.18	0.09	0.00	12.11	640.4	1.830
Lysine	6.4	0.36	0.84	0.12	0.12	0.00	8.72	536.3	1.532
Methionine	5.4	0.25	0.55	0.10	0.05	0.05	7.51	525.8	1.502
Phenylalanine	7.5	0.63	0.77	0.14	0.07	0.00	11.55	678.0	1.937
Threonine	4	0.15	0.34	0.11	0.04	0.00	4.44	376.1	1.075
Tryptophan		0.00	0.00	0.00	0.00	0.00	0.00		0.000
Valine	8.8	0.41	0.90	0.16	0.08	0.00	9.61	573.6	1.639
Arginine	5.9	0.33	0.77	0.11	0.22	0.00	9.58	353.6	1.010
Histidine	2.41	0.13	0.20	0.04	0.07	0.00	3.49	360.9	1.031
Alanine	5.7	0.16	0.37	0.11	0.05	0.00	4.73	377.1	1.078
Asparagine		0.00	0.00	0.00	0.00	0.00	0.00		0.000
Aspartate	9.2	0.34	0.60	0.34	0.09	0.00	11.42	252.4	0.721
Cysteine		0.00	0.00	0.00	0.00	0.00	0.00		0.000
Glutamate	15.7	0.73	1.32	0.59	0.15	0.00	21.54	342.6	0.979
Glutamine		0.00	0.00	0.00	0.00	0.00	0.00		0.000
Glycine	3.2	0.06	0.15	0.06	0.03	0.00	2.24	223.8	0.639
Proline	3.8	0.18	0.32	0.07	0.04	0.00	4.08	535.0	1.529

Serine	8.5	0.24	0.55	0.24	0.08	0.00	8.33	266.4	0.761
Tyrosine	3.75	0.31	0.38	0.10	0.03	0.00	6.33	587.2	1.678
Hydroxyproline		0.00	0.00	0.00	0.00	0.00	0.00		0.000
Hydroxylysine		0.00	0.00	0.00	0.00	0.00	0.00		0.000
Sum	107.26	5.24	10.13	2.62	1.27	0.05	134.37	462.7	1.322

60

61 *Ox bone collagen*

Amino acid	g	n	a	b	c	d	MWt (g/mol)	Bu at STP (mL CH ₄ /g VS)	COD (g COD/g VS)
Isoleucine	1.73	0.09	0.19	0.03	0.01	0.00	1.91	640.4	1.830
Leucine	3.93	0.20	0.43	0.07	0.03	0.00	4.34	640.4	1.830
Lysine	4.11	0.21	0.48	0.07	0.07	0.00	5.06	536.3	1.532
Methionine	0.8	0.03	0.07	0.01	0.01	0.01	1.01	525.8	1.502
Phenylalanine	2.88	0.22	0.27	0.05	0.02	0.00	4.01	678.0	1.937
Threonine	2.52	0.08	0.19	0.06	0.02	0.00	2.53	376.1	1.075
Tryptophan		0.00	0.00	0.00	0.00	0.00	0.00		0.000
Valine	2.65	0.11	0.25	0.04	0.02	0.00	2.62	573.6	1.639
Arginine	9.2	0.47	1.09	0.16	0.31	0.00	13.50	353.6	1.010
Histidine	0.96	0.05	0.07	0.02	0.02	0.00	1.25	360.9	1.031
Alanine	10.9	0.28	0.64	0.18	0.09	0.00	8.18	377.1	1.078
Asparagine		0.00	0.00	0.00	0.00	0.00	0.00		0.000
Aspartate	7.1	0.24	0.42	0.24	0.06	0.00	7.96	252.4	0.721
Cysteine		0.00	0.00	0.00	0.00	0.00	0.00		0.000
Glutamate	11.9	0.50	0.90	0.40	0.10	0.00	14.75	342.6	0.979
Glutamine		0.00	0.00	0.00	0.00	0.00	0.00		0.000
Glycine	25.3	0.43	1.07	0.43	0.21	0.00	16.00	223.8	0.639
Proline	14.7	0.62	1.11	0.25	0.12	0.00	14.26	535.0	1.529
Serine	4.24	0.11	0.25	0.11	0.04	0.00	3.75	266.4	0.761
Tyrosine	0.56	0.04	0.05	0.01	0.00	0.00	0.85	587.2	1.678
Hydroxyproline	14.1	0.59	1.07	0.36	0.12	0.00	15.58	427.1	1.220
Hydroxylysine	1.12	0.06	0.13	0.03	0.02	0.00	1.53	448.9	1.282
Sum	118.7	3.67	7.49	2.13	1.16	0.01	101.99	396.9	1.134

62

63 *Stock medium formulation*

64 **Table S5.** Nutrient and trace element concentrations used in the BMP assays.

Basal medium	Concentration (mg/L)
NH ₄ Cl	200
KCl	100
MgCl ₂ 6H ₂ O	600
KH ₂ PO ₄	138
K ₂ HPO ₄	176
Vitamins	
Yeast extract	100
Trace elements	
FeCl ₃ 6H ₂ O	200
MnCl ₂ 4H ₂ O	4
CoCl ₂ 6H ₂ O	10
NiCl ₂ 6H ₂ O	10
ZnCl ₂ 2H ₂ O	0.5
Na ₂ SeO ₃	0.1
Na ₂ MoO ₄ 2H ₂ O	0.5
CaCl ₂ 2H ₂ O	100
CuCl ₂ 2H ₂ O	0.5
KI	10
H ₃ BO ₃	0.5
Na ₂ S 9H ₂ O	100
Others	
Resazurin	1
NaHCO ₃	4200

Supplementary Results

Table S6. Summary of the observed methane production potentials (B_o) and methanogenic biodegradable fractions (f_D) for the pure biomolecules obtained in the BMP assays.

Carbohydrates	Theoretical (B_{th})	B_o				f_D			
		Mesophilic	SD	Thermophilic	SD	Mesophilic	SD	Thermophilic	SD
Cellulose	414.5	331.3	37.9	359.3	26.2	0.80	0.09	0.87	0.06
Hemicellulose, Xylan	423.9	222.6	49.9	279.1	31.5	0.53	0.12	0.66	0.07
Starch	414.5	191.8	2.1	268.8	12.1	0.46	0.01	0.65	0.03
D(+)-Cellobiose	392.6	359.3	63.2	338.9	40.4	0.92	0.16	0.86	0.10
D(+)-Glucose	373.0	323.9	22.6	313.9	3.3	0.87	0.06	0.84	0.01
Proteins									
Casein	460.4	407.3	31.1	375.5	9.0	0.88	0.07	0.82	0.02
Albumin	462.7	390.0	36.9	354.6	15.7	0.84	0.08	0.77	0.03
Collagen	396.9	302.5	32.0	353.9	4.1	0.76	0.08	0.89	0.01
Lipids*									
Glycerol tripalmitate (GTP)	1005.8	915.0	-	655.7	37.7	0.91	-	0.65	0.04
Glycerol tristearate (GTS)	1023.9	903.9	-	1056.6	-	0.88	-	1.03	-
Glycerol trioleate (GTO)	1011.9	1101.2	-	919.6	72.5	1.00	-	0.91	0.07
Mix GTP-GTS-GTO	1013.9	865.2	50.6	986.9	18.0	0.85	0.05	0.97	0.02

*Blank spaces in SD due to replicates of some lipid samples that did not produce biogas

66 **Table S7.** Biochemical methane potential (mL CH₄/g VS added) and methanogenic biodegradable
 67 fraction (decimal) of lignocellulosic materials.

	Mesophilic				Thermophilic			
	<i>B_o</i>	SD	<i>f_D</i>	SD	<i>B_o</i>	SD	<i>f_D</i>	SD
Dairy manure	227.0	36.5	0.48	0.08	207.1	32.2	0.44	0.07
Red onions	322.2	47.2	0.83	0.12	333.5	55.9	0.86	0.14
Corn silage	309.2	91.2	0.74	0.22	320.2	69.7	0.76	0.17
Wheat straw	285.8	35.4	0.65	0.08	255.0	7.2	0.58	0.02
Miscanthus	256.6	38.1	0.60	0.09	299.5	40.2	0.70	0.09
Switchgrass	238.0	17.3	0.54	0.04	213.3	8.6	0.49	0.02

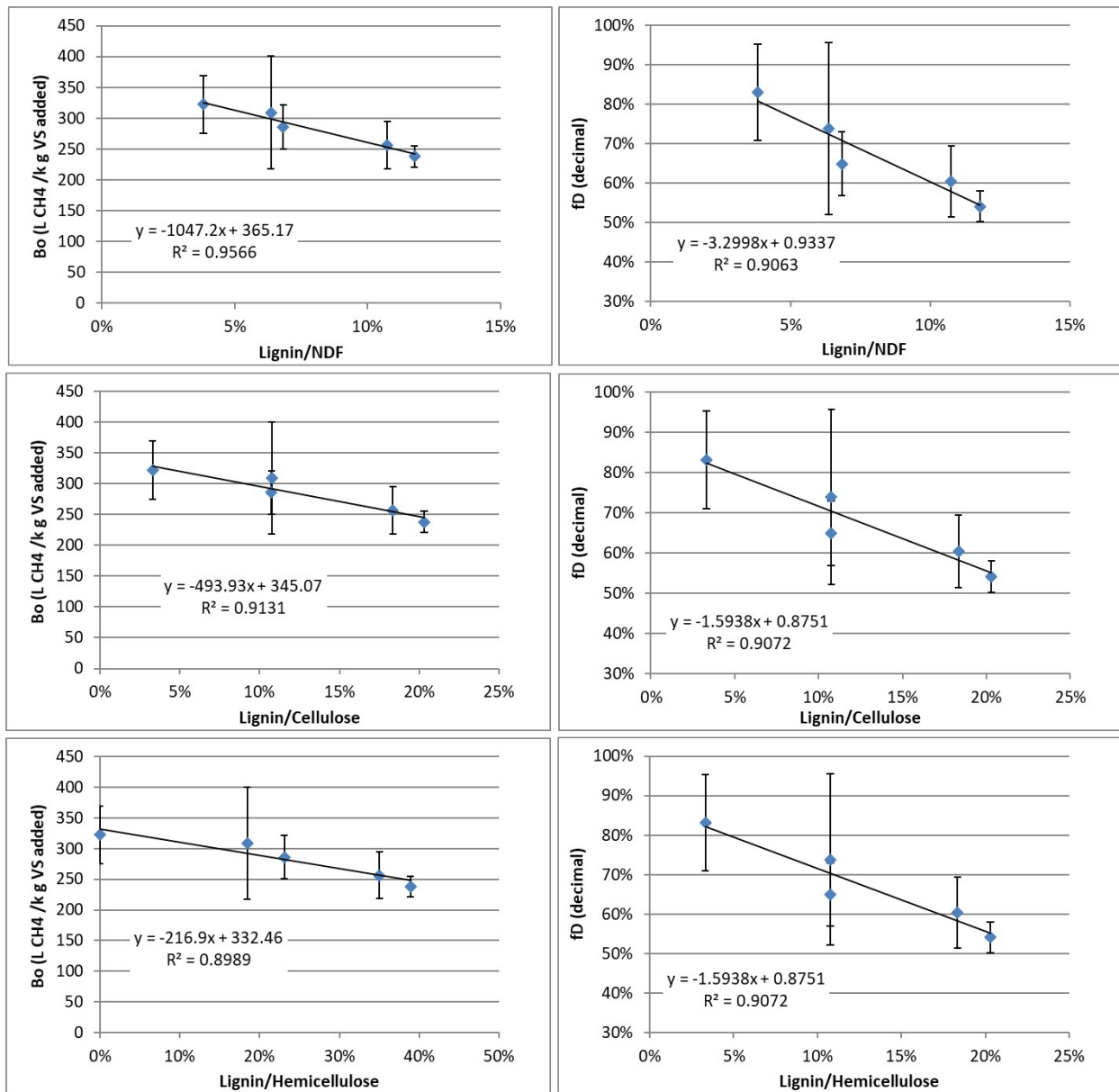
68

69 **Table S8.** Hydrolysis constants (d⁻¹) of lignocellulosic materials.

	Mesophilic		Thermophilic	
	<i>k_h</i>	SD	<i>k_h</i>	SD
Dairy manure	0.21	0.02	0.14	0.06
Red onions	0.44	0.06	0.22	0.02
Miscanthus	0.10	*	0.15	0.05
Switchgrass	0.12	0.03	0.12	0.06

70 * Only one replicate was observed

71



72

73 **Fig. S1.** Correlations between the ratio of lignin to other fiber components and the biochemical methane
 74 potential, B_o – (left column), or the methanogenic biodegradable fraction, f_D (right column).

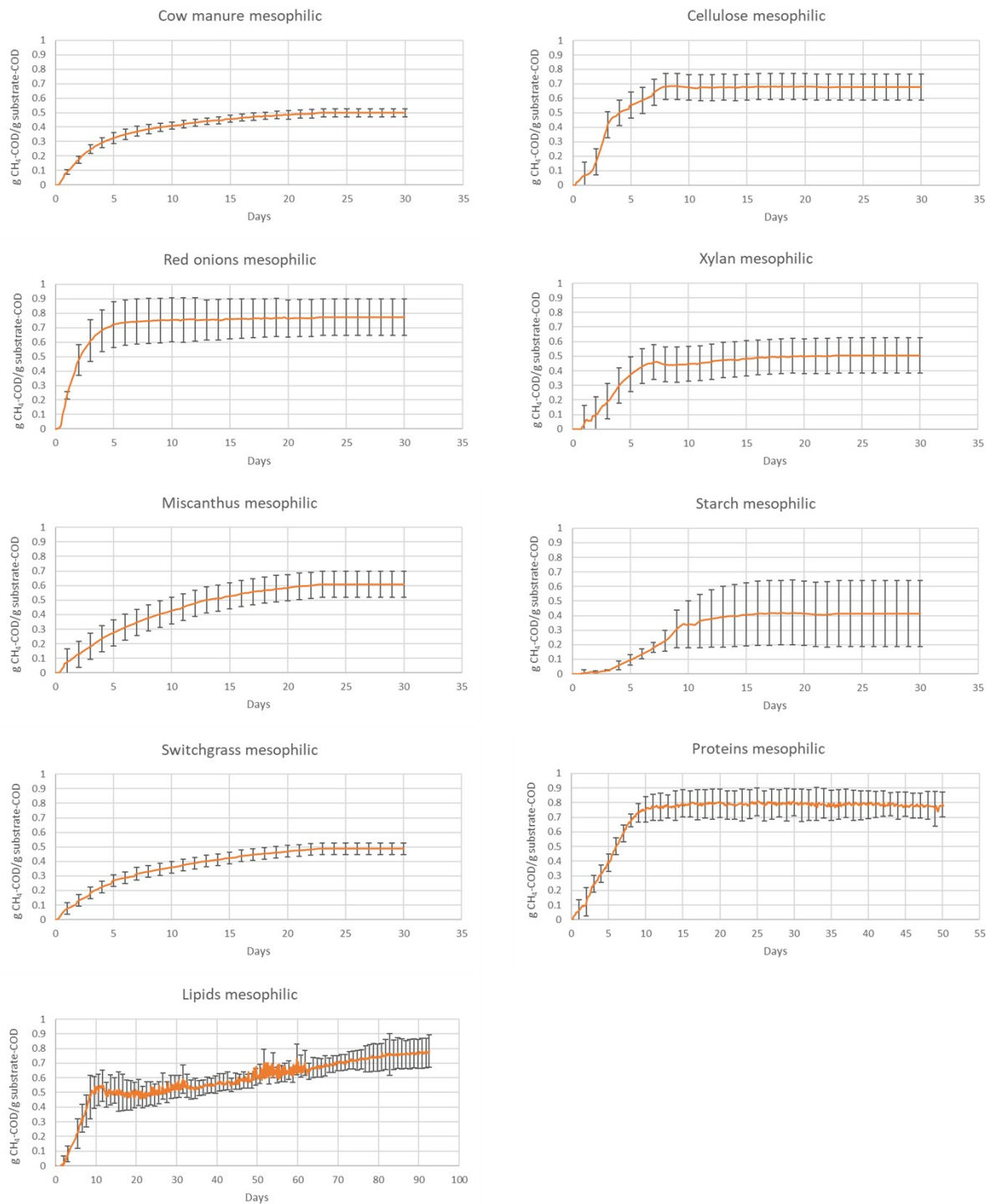
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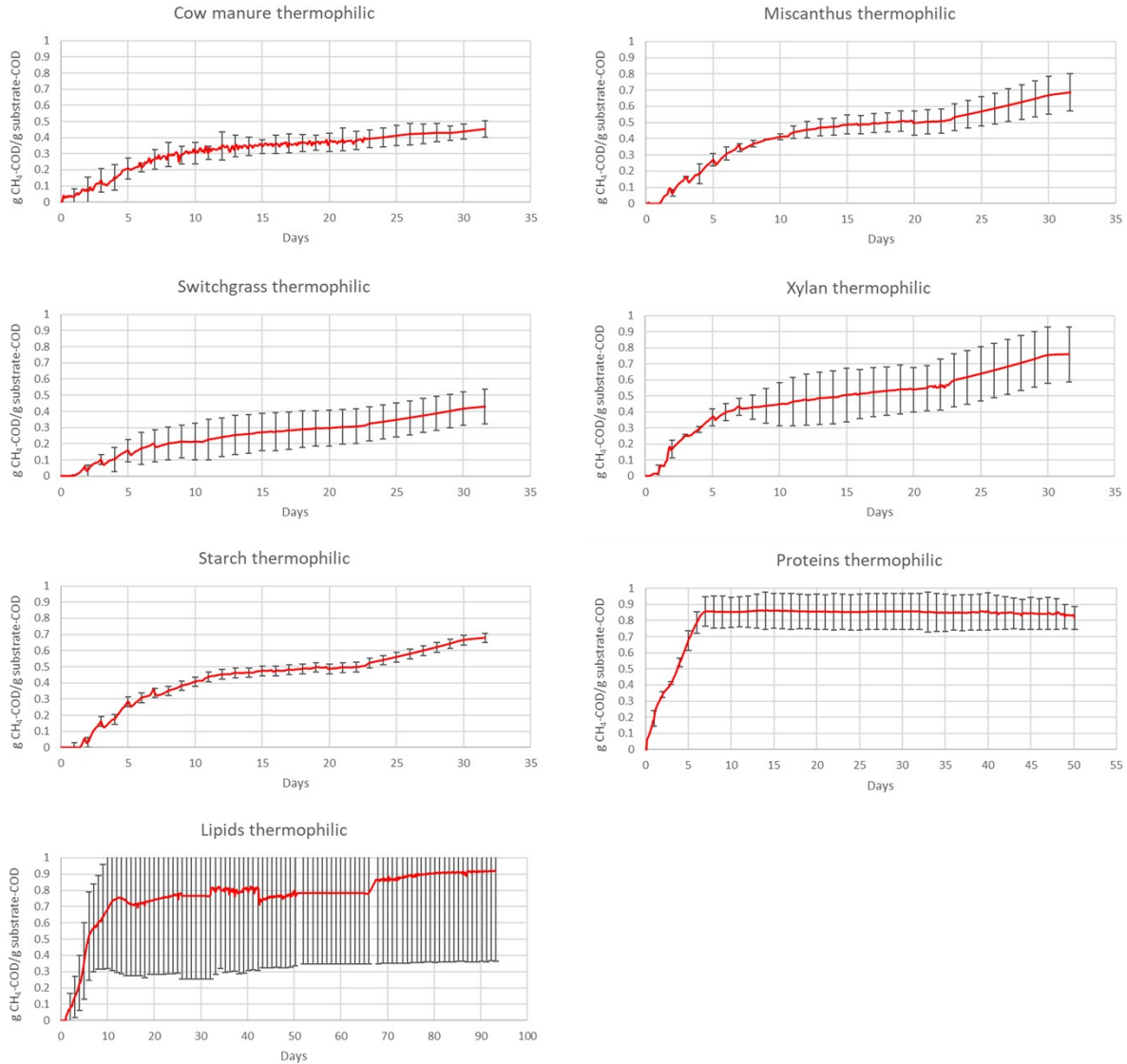
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81 **Fig. S2 (Mesophilic).** Selected* methane production curves (g CH₄-COD/g substrate-COD) as observed
 82 in the mesophilic BMP assays. Some BMP values may differ from those shown on Tables S6 and S7 as
 83 not all the replicates for each substrate were monitored with pressure transducers. *Not all BMP curves
 84 are shown due to faulty pressure transducers, resulting in incomplete continuous data (this did not affect
 85 the ultimate methane production, which was manually measured at the end of the assay).



86

87 **Fig. S2 (Thermophilic).** Selected* methane production curves (g CH₄-COD/g substrate-COD) as observed
 88 in the thermophilic BMP assays. Some BMP values may differ from those shown on Tables S6 and S7 as
 89 not all the replicates for each substrate were monitored with pressure transducers. *Not all BMP curves are
 90 shown due to faulty pressure transducers, resulting in incomplete continuous data (this did not affect the
 91 ultimate methane production, which was manually measured at the end of the assay).

92